Final Report

RESEARCH ON THE PROPERTIES OF CIRCADIAN SYSTEMS AMENABLE TO STUDY IN SPACE

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Figure 1. The little pocket mouse, Perognathus longimembris, implanted with a biotelemeter and ready for insertion in Experiment S-071 Space Experiment Hardware.

PART I

Skylab Experiment S-071

The Effects of Weightlessness on Circadian Periodicity in the Little Pocket Mouse, Perognathus longimembris

I. INTRODUCTION

"Circadian" periodicity represents a class of biological rhythmicity with a period of approximately 24 hours. While the phenomenon has been known for well over a hundred years, its relevance to human well being is fairly recent (1, 2, 3). Many studies have documented the occurrence of circadian periodicity and its role in the temporal organization of life processes, but the exact nature of the "biological clock" has been difficult to establish. Early in the history of the subject, it was observed that circadian periods approximated the length of the earth day; were present in most forms of life; and persisted in organisms "isolated" from cyclic environmental events. These observations raised the question as to whether this kind of rhythmicity was a manifestation of physiological processes that had evolved in an earth environment, or whether there was a circadian "cue" from some pervasive geophysical event that entrained the biological system (4). Today investigators supporting the hypothesis that circadian periodicity is determined by environmental factors are in a minority. Their contention, however, has been difficult to disprove since there are organisms which apparently do not express circadian periodicity unless entrained to environmental stimuli; and it is well known that the environment does modify expression of the circadian period in organisms which do show circadian organization in the absence of environmental stimuli.

Experiment S-071, as originally conceived in 1965, was designed to test the premise that circadian periodicity was dependent on "pervasive geophysical forces." The rationale was that if circadian periodicity persisted in space, where organisms were either divorced from terrestrial cues or sensed them at frequencies other than 24 hours, it would be clear evidence in favor of the physiological, or endogenous, nature of the biological clock. Two factors forced a reevaluation of Experiment S-071 and resulted in a modification of objectives to a study of the effects of weightlessness.

First, research conducted over the last five years has significantly strengthened the argument that circadian periodicity is controlled primarily by endogenous factors and only phased by environmental cues (5). The most compelling evidence results from genetic studies which demonstrated the presence of specific genes responsible for setting the length of the circadian period in Drosophila (6). One allele even results in arrhythmia. Second, the orbital characteristics of Skylab 3 precluded resolution of the possible effects of geophysical factors. It is well established in the literature that circadian periodicity can result from stimuli delivered at frequencies other than 24 hours (frequency demultiplication and multiplication) (7). These frequencies are usually harmonics of a 24 hour period, but some evidence exists for stimuli delivered at 48 hour intervals successfully entraining circadian systems. It can be argued that, if indeed circadian organization is dependent upon geophysical factors, animals close to the earth in a circular orbit with a 90 minute period may still sense some geophysical factor at a frequency capable of maintaining their circadian organization. The argument is tenuous. We have been unable to define, or have defined for us, what geophysical factor or space environment factor could be involved under the circumstances and sensed by an organism housed within the protective shield of space hardware. Interpretation is further complicated by weightlessness.

In our judgement, the question of the endogenous versus exogenous nature of circadian periodicity is no longer a significant enough question to justify the high cost of a space biology experiment. However, we are convinced that knowledge of the way in which circadian systems respond to weightlessness is a significant question which relates both to manned space flight and to the design of future space biology experiments whose results may be predicated on a stable circadian system. It has been shown that an organisms circadian organization can be disrupted (desynchronized) by environmental extremes, and that such disruptions can be detrimental (1, 9). It follows that the behavior of circadian systems in the environmental extreme of weightlessness must be established.

II. EXPERIMENT OBJECTIVES

The objective of Experiment S-071 was to study the circadian system of a mammal during space flight. Specifically, the question asked was whether the periods of the circadian rhythms of body temperature and animal movement in pocket mice would be affected under conditions of prolonged weightlessness? Free ranging mice were to be maintained in space in a closely controlled environment for 30-56 days under conditions of constant dark and constant temperature.

If it was found that the circadian periods were unaffected in either precision or phase relationship by prolonged weightlessness, it would be reasonable to conclude that circadian organization in pocket mice is not dependent on gravity. If the circadian periods degraded, and all other variables were accounted for, it would be reasonable to assume that weightlessness had in some way contributed to the effect (10, 11).

III. METHODS

A. Experiment Organism

The Little Pocket Mouse (Perognathus longimembris) is a heteromyid rodent distributed throughout arid regions of the southwestern United States and Northern Mexico (Figure 1). It is adapted to the extremes of the desert environment in two important ways. First, it does not require drinking water and subsists on air dried seeds and plant material. It meets its water requirement in much the same way as the Kangaroo Rat (Dipodomys) depending both upon resorption of metabolic water and a behavior pattern which reduces evaporative water loss (12). Second, it is a facultative homeotherm with an ability to drop its metabolic rate dramatically while at rest or in response to environmental stress (i.e. ambient temperature, lack of food, confinement) (13, 14, 15). While studying the thermoregulatory behavior of P. longimembris, we noted the precision of the timing of daily drops in body temperature (torpor) and were impressed with the suitability

of the animal for studies of circadian periodicity (16). In addition, since pocket mice do not drink water, animal wastes are concentrated. Less than 0.5 ml/day of urine and 0.1 g/day of feces are produced. Pocket mice are hoarders and food can be provided ad libitum. These traits permit continuous studies of several weeks duration on isolated animals uninterrupted by cage cleaning or animal feeding.

Following preliminary studies with Perognathus parvus, and Perognathus formosus (Appendix A), Perognathus longimembris was selected for Experiment S-071. The reasons were: first, a well defined marker of a circadian phenomenon (torpor); second, the ease of maintaining the animal during a space experiment; and third, its small size (~ 10g) which was conducive to a statistically significant sample size.

B. Biotelemetry

A small blocking oscillator type transmitter was developed (Ref. 17 and Appendix B). The pulse rate of the transmitter was determined by a variable resistor (thermistor) whose resistance was proportional to temperature. The transmitter was powered by a 1.35V nickle-cadmium battery and the entire assembly encapsulated in paraffin. Ready for insertion into the abdominal cavity of the mouse, the transmitter weighed approximately 1.3g, measures 1.8 x 0.5 x 0.9 cm, and had an operating expectancy in the mouse of six to nine months. Figure 2 shows body temperature data obtained with this transmitter along with one way of quantitatively determining the circadian period of a mouse expressing daily torpor. We arbitrarily chose the midpoint of arousal from torpor as the phase reference point. When the time of occurrence of the phase reference point was plotted for successive days and a curve fit by the method of least squares, a quantitative expression of both period length and its precision was obtained which was in good agreement with other methods of time series analysis (18, 19, 20).

We also measured the circadian characteristics of animal movement specifically looking for evidence of changes in phase relationship between the circadian period of body temperature and activity (20). The method of

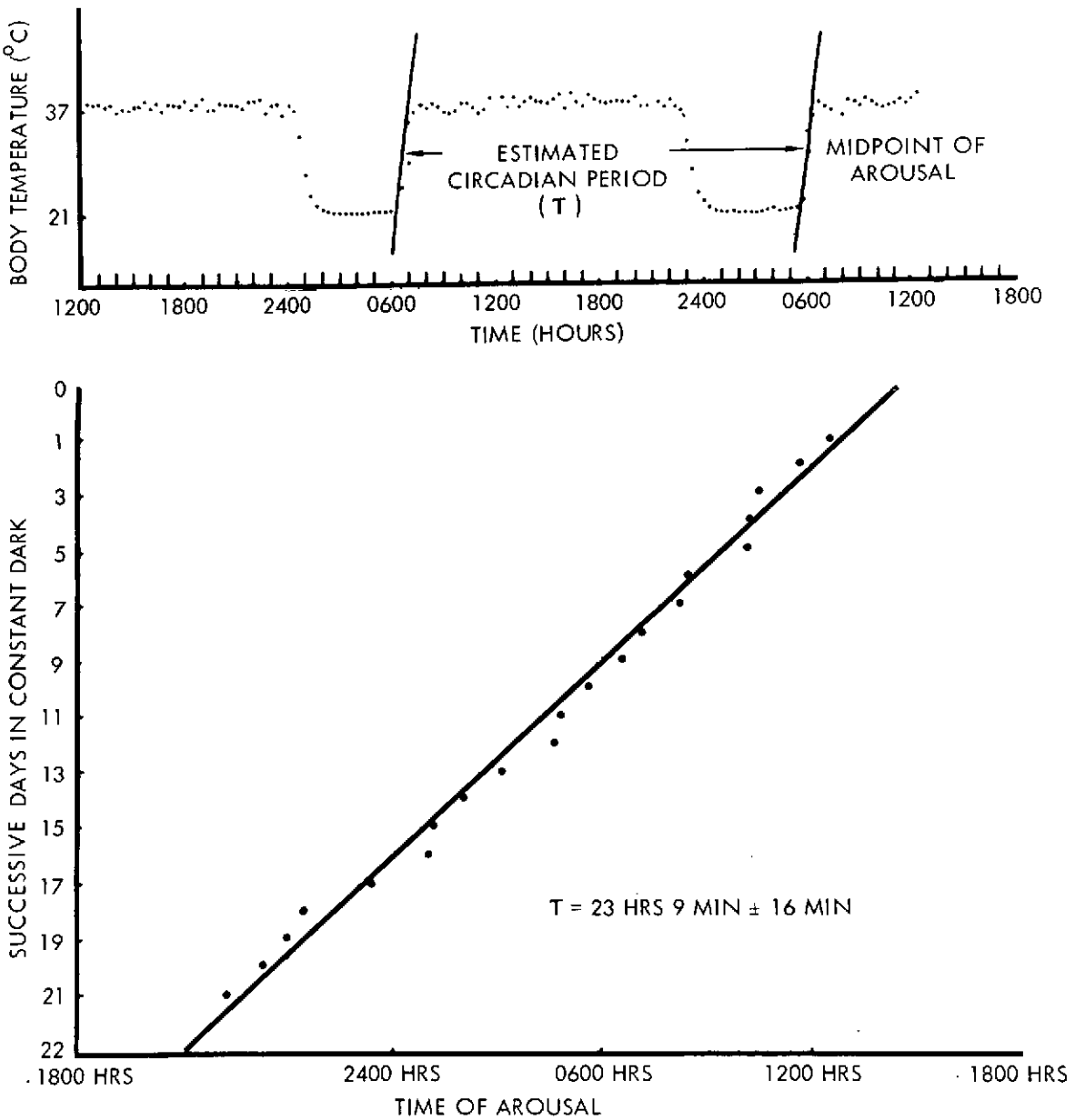


Figure 2. Estimation of the free-running circadian period (τ) of body temperature in Perognathus longimembris. Body temperature measurements taken at 10 minute intervals over a period of 2 days are plotted in the upper portion of the figure. Time between midpoints of successive arousals from torpor is an estimate of τ. Times of arousal from torpor on successive days are plotted in the lower figure. A curve fit by the methods of least squares to times of arousal yields an estimated $\tau \pm$ S. D.

monitoring activity capitalized on the fact that as the animal moved, the implanted transmitter also moved and changed its relationship with the receiving antenna. This movement resulted in a change of signal strength. A measure of relative animal activity was obtained by scoring the number of changes in signal strength in each 10 minute period. Electronically there are several ways of accomplishing the feat. The method used in Experiment S-071 is diagrammed in Figure 3.

In effect telemeter movement was monitored and any action on the part of the animal which results in telemeter displacement was detected. When the animal was at rest, breathing rate could be detected easily and, in some circumstances, even heart rate. Considerable empirical experimentation was required to adjust the threshold of the detection circuit to score only physical displacement of the mouse.

The activity detection system was calibrated in two ways. First, activity of an instrumented mouse was monitored simultaneously by telemeter displacement, and by an acto-ballistocardiograph supplied by NASA/ARC (21). The two activity records, while not identical, were so similar that it was difficult to identify the monitoring system from unlabeled records. Secondly, an instrumented mouse was monitored simultaneously by telemeter displacement and by running wheel activity. Again, the similarity between records was striking (Figure 4). Our conclusion was that the telemeter displacement method of monitoring pocket mouse activity would produce useful data of a quality similar to alternate monitoring methods.

One drawback of the telemeter displacement method of monitoring activity is that it cannot discriminate between different kinds of behavior. Thus, a high activity count can result from exploratory motions, preening, digging, running, etc., either singularly or in combination. An example is the burst of activity coincident with arousal from torpor. This activity is associated with the rewarming process while activity burst later in the day may be associated with feeding.

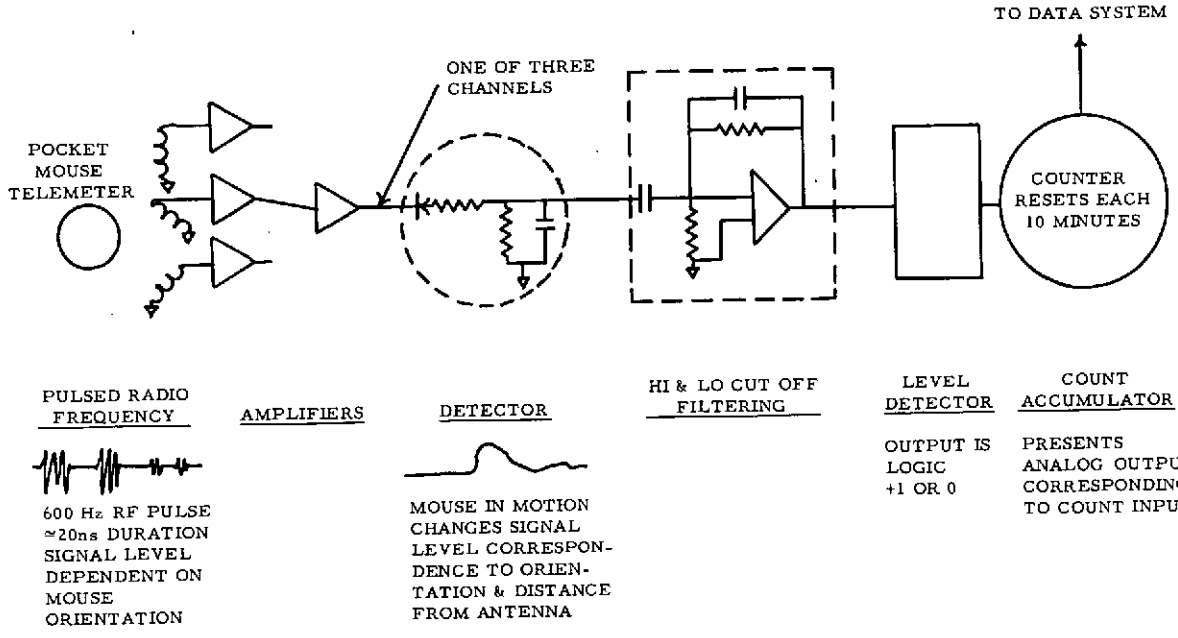


Figure 3. Diagramatic representation of electronic circuitry used in Experiment S-071 space experiment hardware to detect and score animal movement. Principle of detection was based on changes in signal strength produced by movement of the biotelemeter.

A second weakness of the method is that every significant movement is not necessarily scored. As a result the recorded activity represents a relative value difficult to quantitate. The problem is further complicated by the difficulty of adjusting the detection circuitry to guarantee similar sensitivity from cage to cage in order to permit comparisons between individual animals. Recognizing those problems, it is almost surprising that the data in Tables IV and V show as good agreement as they do.

In spite of the potential drawbacks we were struck by the fact that onsets and cessation of major activity (commonly used markers of circadian behavior) were easily determined by the telemeter displacement method. Further, the resultant estimate of circadian period compared favorably to circadian periods determined by running wheel activity (Figure 4).

Although a successful means of monitoring activity, the telemeter displacement method was probably an unwise engineering choice for Experiment S-071. The least reliable component in the monitoring system was the battery used to power the telemeter. If the telemeter had failed, both body temperature and animal activity would have been lost. Independent monitoring systems for temperature and activity would have increased the probability of successfully monitoring at least one of the biological parameters. In general we were well satisfied with the activity monitoring and would recommend its consideration to other experimenters. If telemetry is already being used for such parameters as body temperature, EKG, etc., it would be relatively simple to also obtain data on activity patterns.

C. Space Experiment Hardware

The Major elements of Experiment S-071 hardware were an animal enclosure, environmental control system (ECS), electronics and power supplies, and a data handling and storage system (CDS) which was shared with Experiment S-072 (22). Experiments S-071 and S-072 shared similar scientific objectives and were packaged as an integrated unit which interfaced with the spacecraft for power, thermal control, data retrieval and command functions, and mounting in Bay I of the Command Service Module (CSM) (Figure 5A).

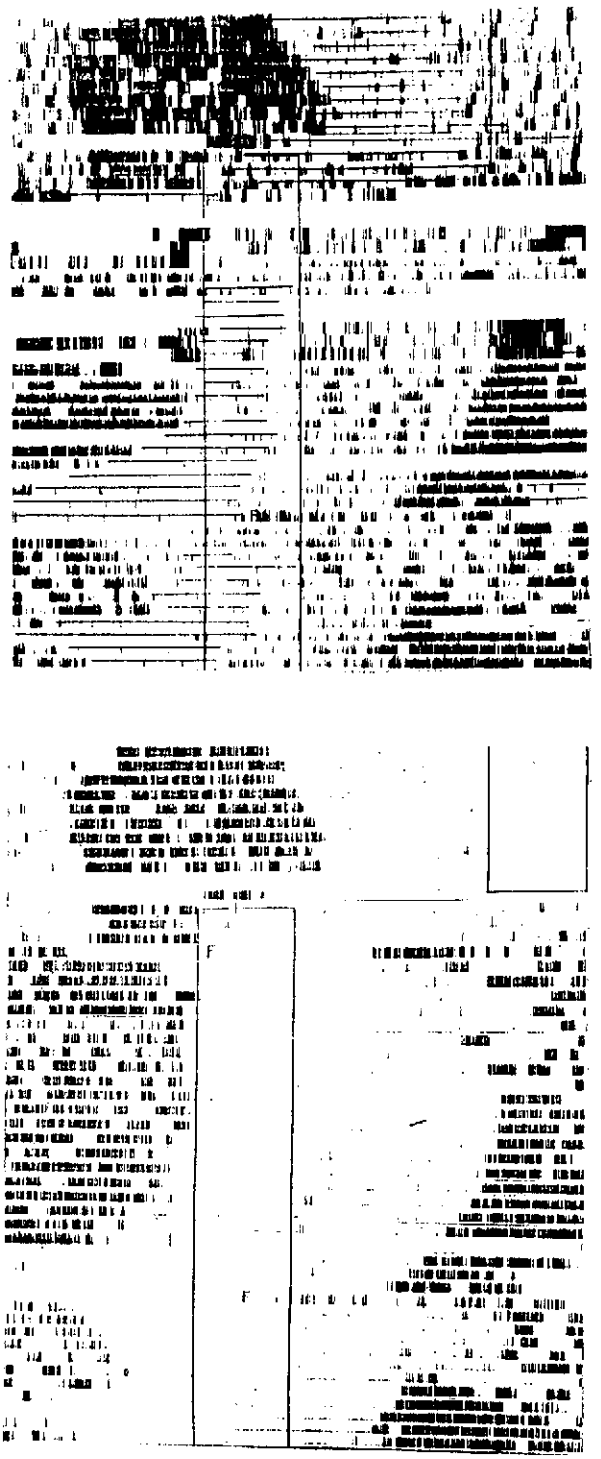


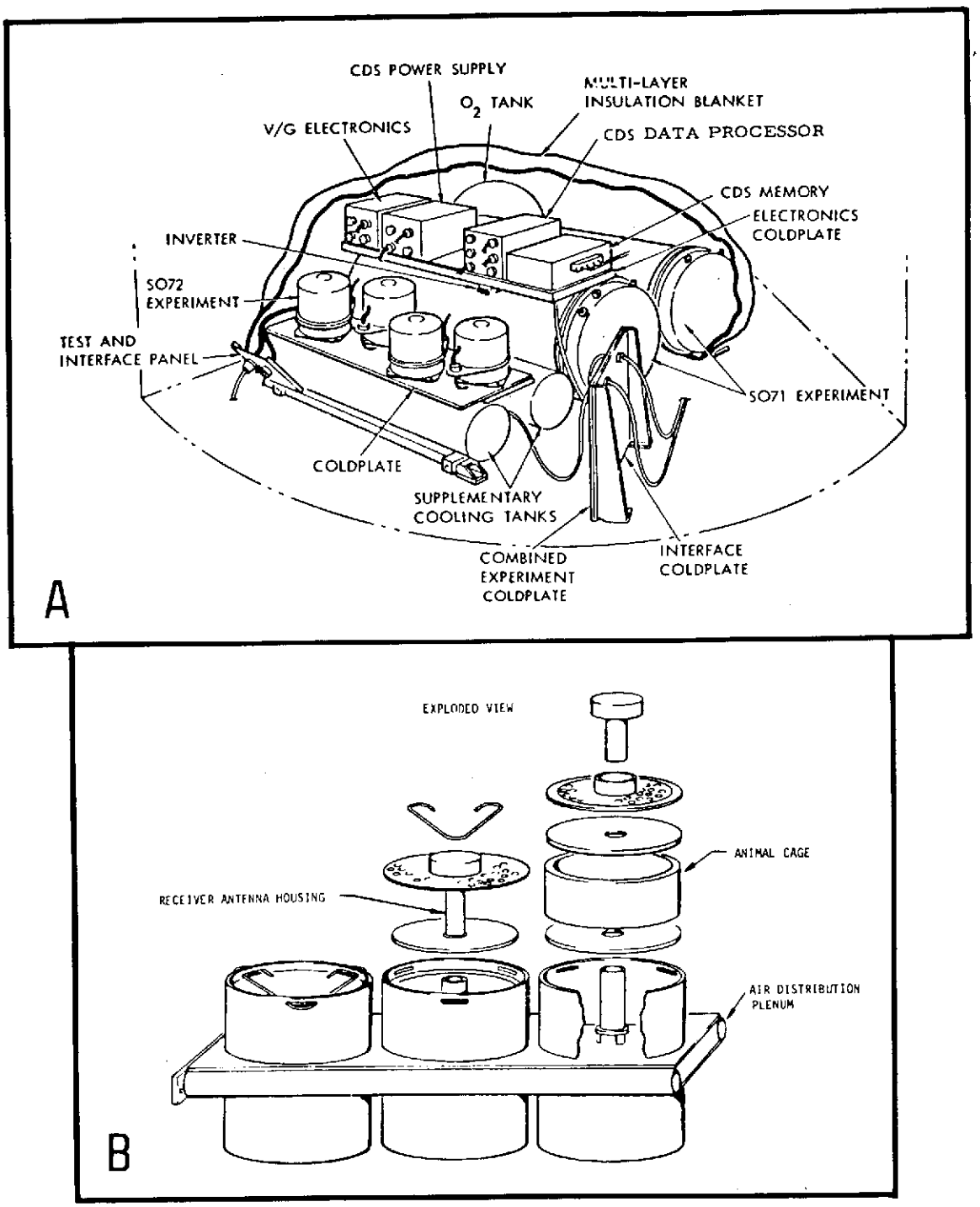
Figure 4. Comparison of Methods for Monitoring Animal Activity.

Upper: Telemeter Displacement
Lower: Running Wheel

Both records derived simultaneously for 50 days. Vertical box indicates light regimen (230 lux). Solid horizontal line each day indicates time in torpor. "F" indicates time of adding food.

Figure 5. Space experiment hardware configurations. (A) Hardware configuration for the combined Experiments S-071 and S-072. The inner cylinder, labeled Experiment S-071, contains the pocket mouse cages. The outer cylinder is the environmental control system for Experiment S-071. The data processing and storage system (CDS) was shared by both experiments.

(B) Configuration of the cage/air plenum assembly slides into the cylinder described above. For further information see reference 22.


CDS POWER SUPPLY
O_2 TANK
MULTI-LAYER INSULATION BLANKET
V/G ELECTRONICS
CDS DATA PROCESSOR
CDS MEMORY ELECTRONICS COLDPLATE
INVERTER
SO72 EXPERIMENT
TEST AND INTERFACE PANEL
SO71 EXPERIMENT
COLDPLATE
SUPPLEMENTARY COOLING TANKS
COMBINED EXPERIMENT COLDPLATE
INTERFACE COLDPLATE
A
EXPLODED VIEW
ANIMAL CAGE
RECEIVER ANTENNA HOUSING
AIR DISTRIBUTION PLENUM
B

The animal enclosure was a circular tank containing six cages mounted on a common air distribution plenum, and antennas and receivers (Figure 5B). Each cage was 15 cm in diameter, 4 cm high, lined with porous polyethylene, and contains one instrumented mouse and 50g of air dried seeds. In the center of each cage was a 2.5 cm diameter fiberglass tube enclosing the receiving antennas and supporting the receiver. A circuit for scoring animal movement was an integral part of the receiver which provided both body temperature and animal activity data to the CDS. Conditioned air was supplied to the plenum from the ECS where it was distributed uniformly to each cage. The air was directed from the cage subplenum through the porous floor and ceiling of the cage at a rate of 85 liters/min into the animal enclosure for return to the ECS. Ambient cage air temperature was monitored by a thermistor placed in the subplenum of each cage.

The Environmental Control System maintained an oxygen nitrogen atmosphere at 700 ± 15 mm Hg, 20 ± 10% R. H. It was a demand type oxygen supply system in which the volume of carbon dioxide and water vapor absorbed was replaced from an oxygen reservoir (Figure 6). The circular ECS tank housed a fan, a charcoal-lithium hydroxide absorption canister, a dew point control heat exchanger, a moisture separator and a temperature control heater. Accessories mounted exterior to the ECS tank included an oxygen supply bottle and pressure regulators; a coolant pump and control elements; and plumbing for connecting the heat exchanger to the spacecraft coldplate. Two supplementary coolant tanks were provided for intermittent use during periods when the spacecraft coldplate exceeded specified limits. The entire S-071/S-072 experiment package was surrounded by a multilayer blanket of gold coated mylar.

The data system (CDS) consisted of a data processor, memory, and power supply. The CDS scanned all biological, environmental, and engineering parameters for both Experiments S-071 and S-072. For Experiment S-071 one body temperature reading and one accumulated activity count for each animal was recorded every 10 minutes for the duration

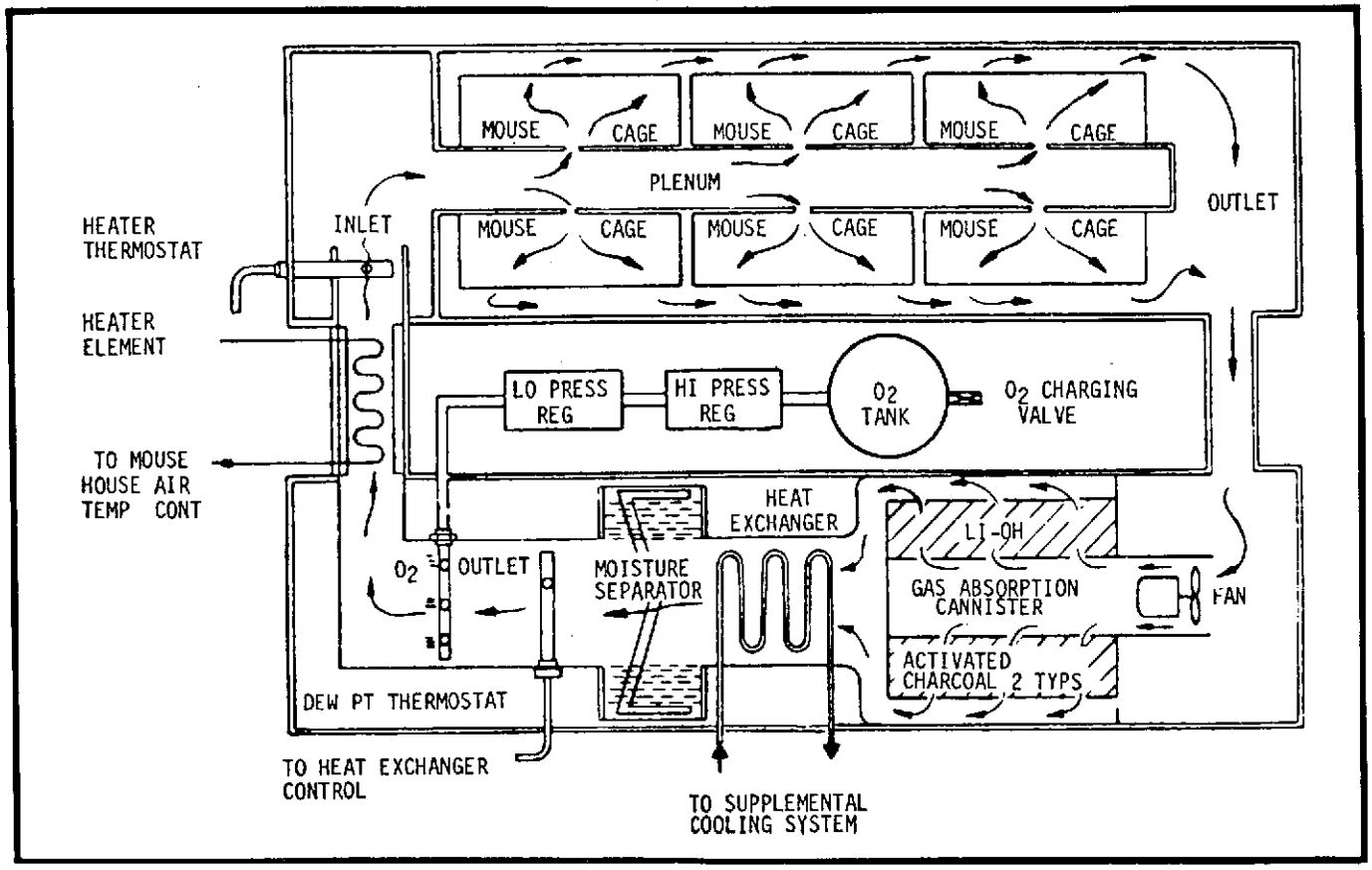


Figure 6. Environmental control system schematic for Experiment S-071. For further information see reference 22.

of the experiment. Ambient air temperature in each cage was also monitored every 10 minutes and other engineering data were monitored once every forty minutes. The CDS stored the data in a core memory and on command transmitted the data to ground stations. It also implemented all commands, generated timing and control signals, and could transmit real time data without disrupting data storage. Experiment S-071 normally required only two commands; first for data dumps, and second, to update the clock. Power could be removed at the end of the experiment or in emergencies through a manual switch in the command module.

D. Animal Holding Unit/Laboratory Monitoring Unit (HU/LMU)

Special experiment support equipment was fabricated under a separate contract. The purposes of this equipment were to provide a facility for obtaining base line data from mice contained in simulated space experiment hardware; a holding facility for mice at the launch site; and to serve as an experiment "control" apart from animals housed in space experiment hardware.

The equipment consisted of three units (Figure 7A). An Animal Holding Unit (HU) consisted of four drawers, each containing six cages with the same configuration and electronics used in the space hardware. A Laboratory Monitoring Unit (LMU) provided signal processing and produced both a digital and magnetic tape record of data collected. An air conditioning unit provided air to the HU at the same temperature, humidity, and flow as specified for the space experiment hardware. The only difference between the mouse-hardware interface of the HU/LMU and space experiment hardware was that the air supply was not recirculated in the HU/LMU. A measure of the effectiveness of the simulation is indicated in Figure 8 which shows the similarity of data collected from the same animal contained first in the HU/LMU and then in space experiment hardware.

The HU/LMU was used extensively to support space experiment hardware development, laboratory research, and execution of the space experiment.

E. Experiment Procedure

1. Selection of Mice for Experiment S-071. The advantages of selecting Perognathus longimembris for Experiment S-071 were complicated by dependency on field collected animals. A random breeding population produces a wide variety of individuals. All mice studied showed circadian organization but the precision of the circadian periods could be highly variable, as could expression of torpor, or behavior patterns such as wheel running, seed sorting, nest building, etc. These variations were of considerable interest to laboratory investigations but it was obvious that for Experiment S-071 animals with well defined circadian rhythms and behavior were needed in order to recognize changes that might be brought about by space flight. As a consequence, a large collection of animals (~500) ranging in age from 1 to 3 years was established. This collection was routinely observed noting the occurrence of torpor, body weights, and tendency to maintain a well ordered cage. Based on these observations, animals were selected for detailed studies on the precision of their circadian periods.

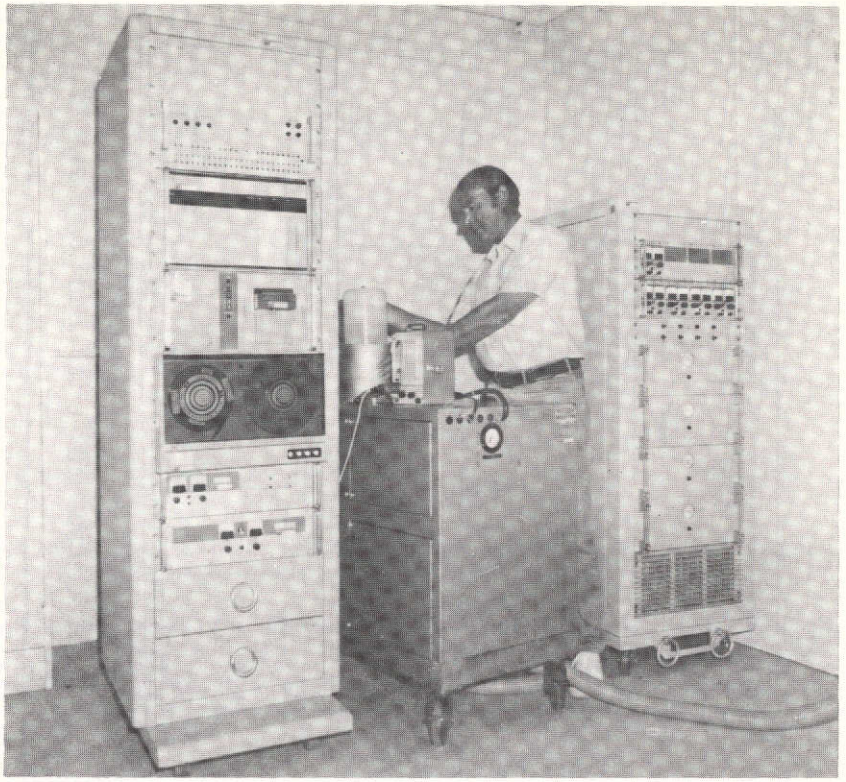
Successive groups of 24 mice implanted with biotelemeters were placed in the HU/LMU and their thermoregulatory behavior, activity, and precision of the circadian period monitored for 21 days. Criteria for selecting candidate flight animals were

- Expression of torpor
- A precise circadian period of body temperature
- Stable or increasing body weight
- A minimum body weight of 10 grams with implanted biotelemeters
- Food consumption
- Seed sorting behavior and general "housekeeping"
- General appearance

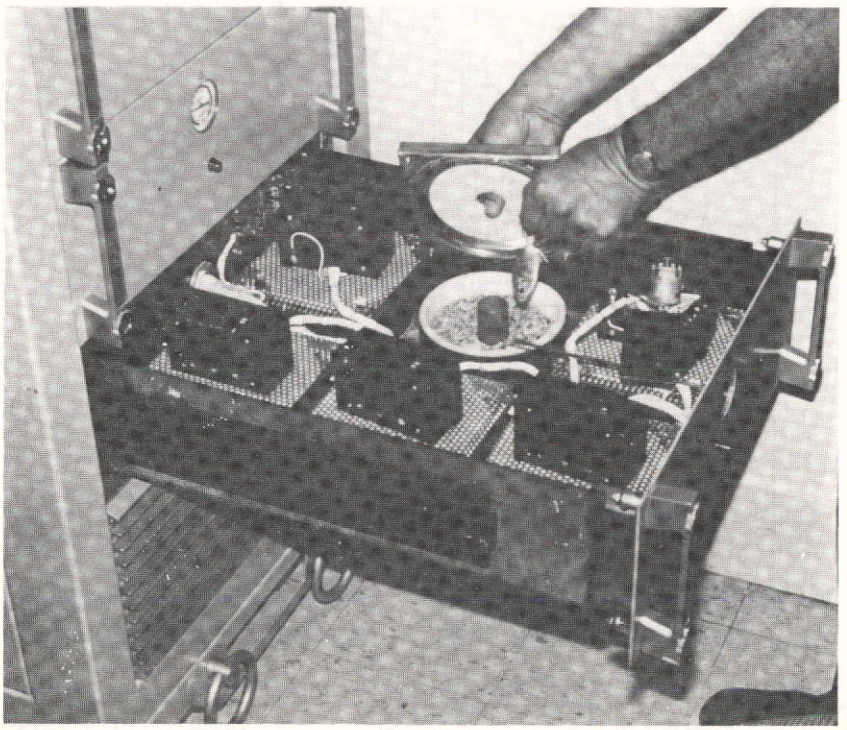
Final screening began in the Fall of 1972 and continued through March 1973. Fifty animals were identified as acceptable flight candidates.

Figure 7. Animal Holding Unit/Laboratory Monitoring Unit (HU/LMU) used in support of Experiment S-071. (A) An air conditioning unit (center) provided air, of the same specification used in the space experiment hardware, to the animal holding unit (right). The animal holding unit consisted of four drawers each containing six cages of the same configuration used in the space experiment hardware. Signals from receivers on the cages in the HU were collected and processed in the laboratory monitoring unit (left) to produce both digital and magnetic tape recordings.

(B) One drawer of the animal holding unit open showing arrangement of cages.



A



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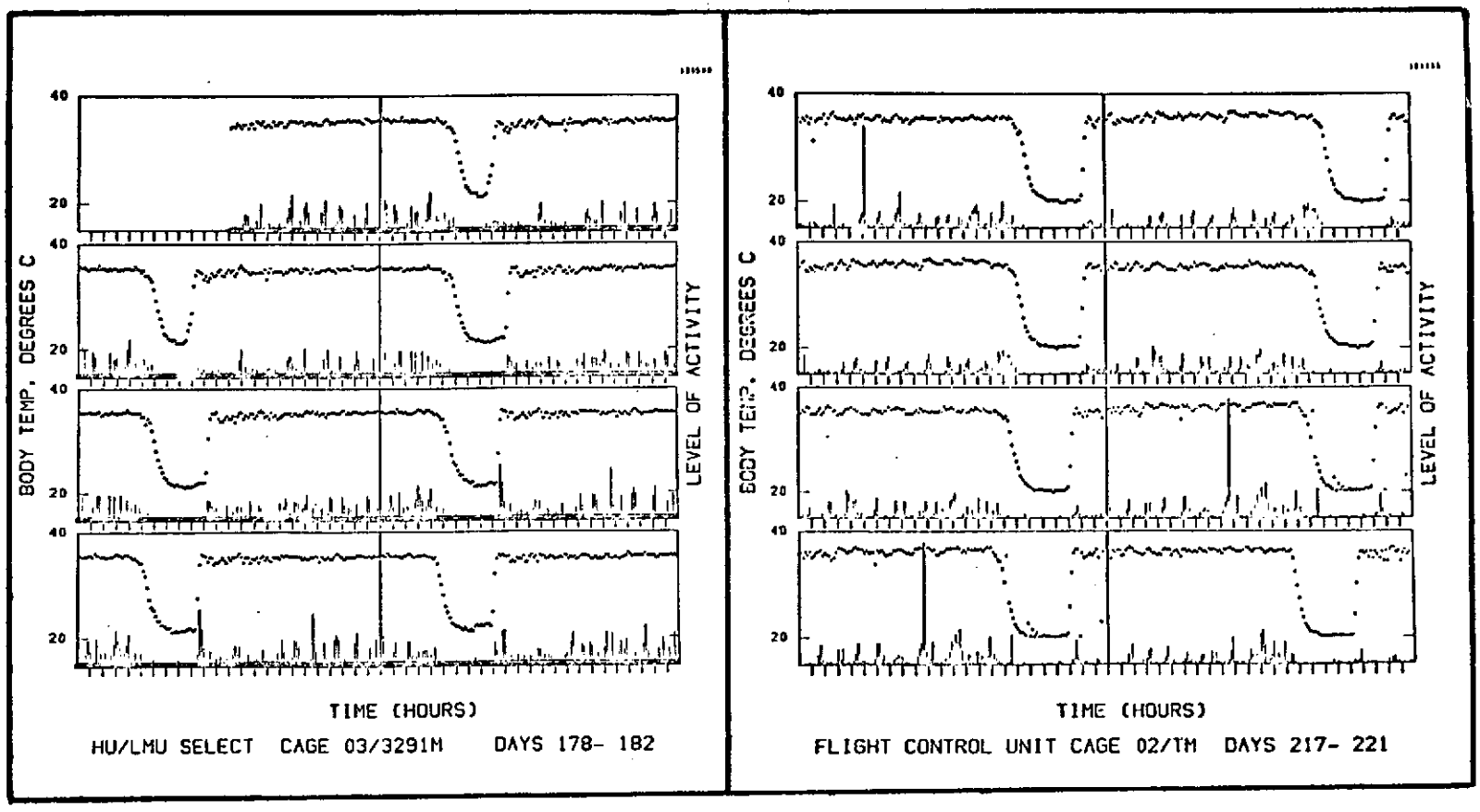

Figure 8. Computer plots of five days of body temperature and animal activity data collected from the same animal at different times in the HU/LMU and Flight Unit 1. Data are double plotted. Time scale equals 48 hours.

2. Biotelemeter Implants. Twenty-eight selected pocket mice were implanted with biotelemeters between 4 June and 6 June 1973.

3. Transport to NASA/KSC. Twenty-eight instrumented mice were transported by air on 18 June 1973 in shipping cages supplied by NASA/ARC. No attempt was made to maintain a constant dark environment. Twenty-four animals were installed in the HU/LMU immediately upon arrival at KSC on 18 June. The four remaining animals were stored in plastic cages for emergency use.

4. Pre-Launch Screening of Animals. Data were collected from 24 animals in the HU/LMU from 18 June through 13 July 1973. The mice were removed from the HU/LMU, weighed, and placed in individual gallon jars containing seed and sawdust bedding. The jars were then placed in a light tight storage box. All manipulations occurred under a dim red photographic safelight. The air conditioning unit used to supply air to the HU/LMU was used to ventilate the storage box. The atmosphere was monitored continuously in the box and judged to be comparable to that experienced by the mice while in the HU/LMU.

Data collected from the HU/LMU in the form of a magnetic tape was processed by the KSC computer center. A calibrated listing of data, plots of body temperature and animal activity, and estimates of τ by autocorrelation were returned to the Principal Investigator by 20 July 1973. Twelve animals were selected for Experiment S-071 on 22 July 1973.

5. Loading of Flight Hardware. The space experiment hardware units designated (CPE 1 and CPE 2) were loaded with six animals each on 22 July 1973. All manipulations occurred under a dim red photographic safelight. Animals were weighed prior to loading, and operation of the biotelemeter was verified. The remaining animals were returned to the HU/LMU.

6. Installation of Space Experiment Hardware in Spacecraft. On 24 July 1973, the second space experiment hardware unit (CPE 2) was placed on a transportation cart at the launch site laboratory and transferred to the spacecraft. The transfer cart provided both power and cooling until these functions were taken over by the spacecraft. It was intended that the mice in both CPE 1 and CPE 2 experience the same constant environment except for the unavoidable noises and movements associated with transferring and mounting CPE 2 in the Command Service Module (CSM). Both units and the HU/LMU were operated continuously from the time the animals were loaded on 22 July 1973 until completion of the mission.

7. Launch and Space Performance. Launch occurred on 28 July 1973 carrying CPE 2. CPE 1 and the HU/LMU were maintained in the laboratory as ground controls. CPE 2 operated perfectly for 30 hours following launch. At that time a power failure resulted in loss of the experiment.

8. Post Launch Activity

(a) Since the reason for the power failure was unknown, and since there was a reasonable chance of repeating the experiment on a later flight, the decision was made to continue to operate the ground controls (CPE 1 and the HU/LMU). It was hoped that either the reliability of the experiment hardware could be reestablished, or the reason for failure of CPE 2 would become apparent.

(b) Ground controls (CPE 1 and the HU/LMU) were operated continuously from 22 July to 20 August 1973. Gas samples were taken from CPE 1 on 18 and 20 August. Magnetic tape records of collected data from both the HU/LMU and CPE 1 were reduced as in Item 4 above.

(c) Upon securing the experiment, the animals were removed, weighed, a photographic record made of the condition of the cages, and an estimate made of the food consumed. The mice were returned to the gallon jars and ultimately returned to NRTC.

9. Securing Launch Site Operations. Laboratory activities at KSC were terminated the week of 27 August 1973.

10. Data Retrieval. A digital print-out of data from the control units was displayed concurrently with magnetic tape recording in the launch site laboratory. Data from the space experiment was retrieved through the NASA communication net from periodic data dumps of the core memory. Near real time data was displayed daily at the Mission Control Center at NASA/JSC to ensure integrity of retrieved data (i.e. drifts in the time code generator), and operationally to determine hardware status as it related to the overall mission. Scientific analysis and evaluation of the experiment was derived from a master magnetic tape of all data retrieved from Experiment S-071 and as necessary examination of mission voice tapes.

IV. RESULTS

A. Performance of Experiment Hardware

The performance of biotelemeters implanted in animals between 4-6 June 1973 is summarized in Table I. Five reference telemeters maintained in a water bath at 38°C between 20 June and 27 August were recalibrated and checked for shifts in center frequency the week of 8 October 1973. While slight changes were observed, they were judged to be in the range of instrument and reading errors.

The HU/LMU operated reliably for the duration of the launch site operations. On one occasion a faulty relay in the air conditioning unit resulted in loss of humidity control for a few hours. It is not apparent from the biological data that this malfunction was sensed, or affected, the end points being monitored.

Definitive statements regarding the functioning of CPE 1 and 2 are reported elsewhere (22, 23). From the standpoint of its adequacy in maintaining a specified experiment environment, and adequately monitoring mouse body temperature and activity, the following observations are pertinent.

TABLE I

BIOTELEMETER PERFORMANCE

Telemeter Number	Date Implanted	Date Failed or Explanted	Effective Life* (Months)
62	4 June 73	7 Dec 73	7.5
63	"	19 Feb 74**	> 10.0
66	"	21 Dec 73	8.0
72	"	21 July 73**	-
77	"	21 Dec 73	8.0
78	"	7 Dec 73	7.5
81	"	20 Aug 73**	-
90	5 June 73	3 Nov 73	7.0
93	"	7 Dec 73	7.5
94	"	7 Dec 73	7.5
96	"	7 Dec 73	7.5
99	"	7 Jan 74**	> 8.0
101	"	21 Nov 73	7.0
102	"	21 Dec 73	8.0
103	"	21 Dec 73	8.0
106	6 June 73	21 Dec 73	8.0
109	"	8 Jan 74**	> 8.0
110	"	4 Dec 73	7.0
111	"	8 Jan 74	> 8.0
112	"	17 Dec 73	> 7.5
113	"	25 July 73**	-
114	"	21 Dec 73	8.0

*Estimated from time of battery attachment (1 May 1973) until removal from mouse.

**Date of explant. Telemeter still functioning

1. Quality of the data were excellent with data loss well below the specified allowable limit (5%).

2. Data collected from the HU/LMU and CPE 1 and 2 were of comparable quality. It was impossible, in scanning unlabled plots, to tell from which equipment the data had been collected. In general, however, the activity records from the Flight Units were more consistent (Figure 8).

3. Thermal control in the Flight Units was excellent and well within specifications.

4. Gas analyses done on the canister atmosphere at the beginning and end of the experiment indicated excellent control of atmosphere quality with one notable exception. On the order of 180 ppm of carbon monoxide was detected after 28 days of continuous operation. The source of this contaminant was later traced to outgassing of the activated charcoal used to scrub the air stream. Carbon monoxide was found in high concentration in the container in which the activated charcoal supply was stored. We assume, therefore, some human error in the storing and/or preparing of the activated charcoal at the vendor's site. It is not apparent that the level of CO observed in the experiment hardware affected the animals. Whether the concentration would have increased or affected the animals on a 60 day mission is a moot question.

5. Shortly after loading the Flight Units with animals on 22 July, CPE 1 was noticed to have a "noisy" coolant pump. As a consequence, CPE 2 was selected for space flight. As the pump on CPE 1 became "noisier," the decision was made to replace it with a spare. The spare pump operated continuously for about 26 days until completion of the experiment on 20 August. The spare pump also became "noisy" a day or two prior to termination, but did not fail.

B. Biological Data

1. Of the twenty-eight pocket mice carried to KSC, three died. One had been used for demonstration purposes and dropped on the floor. It died a few days later from internal hemorrhage. A second mouse became hyperactive, lost large amounts of hair, and weight during the pre-launch study. The remaining mice exhibited stable weights and appeared in prime condition for the duration of their use. Nineteen mice were returned to NRTC. All were alive at time of removal of the biotelemeters (Table I).

2. Given a group of highly screened animals, final selection of mice for Experiment S-071 was based on (a) precision of the free running period in the pre-launch selection study, (b) body weight, and (c) performance during screening studies at NRTC. An example is given in Figure 9. Since laboratory research indicated that τ could be quite variable under different circumstances, more weight was given to the precision of τ than its absolute value. Dispersion values were the principle measure of precision. A summary of the various τ values obtained from animals selected for Experiment S-071 is given in Tables II and III.

3. A history of torpors expressed by mice delegated to CPE 2 is given in Figures 10-15. Figures 16, 17, and 18 are computer plots of body temperature data plus animal activity data collected from the six animals launched on SL-3. The records encompass the time from which the experiment hardware was turned over to the launch support crew until the power failure 30 hours into the mission. Fairly regular bursts of activity at approximately 60-90 minute intervals are apparent for most animals prior to launch (see Figure 17, Cage 4). Further, the bursts of activity correlate with rises in body temperature. Following launch, the tendency for bursts of activity persists but the pattern was more random. Cage 5 (Figure 18) is notably in that the level of activity rises strikingly following launch. We interpret this apparent increased activity as real. The assumption is supported by the body temperature which is unusually high and

uniform during high activity readings and tends to drop as activity decreases. The very low activity detected in Cage 2 (Figure 16) may reflect an improper setting of the detection circuit. Similar data from the six animals in the ground control unit were analyzed. The period chosen for analysis was the 21 days preceding termination of the ground control. Table IV summarizes the activity characteristics noted in the ground control animals and Table V summarizes similar characteristics of the flight experiment animals for the three days prior to launch.

Tables IV and V were derived by averaging the daily activity record referenced to time of arousal from torpor. For example, a summary plot of four days of data collected from Cage 4 (Figure 17) prior to launch is presented in Figure 19. The first burst of activity, coincident with arousal from torpor, is associated with thermoregulation. The next major burst occurs (in the case of this animal) at about four hours following arousal and marks onset of major activity. Onset of major activity as detected by this monitoring system corresponds to onset of running wheel activity in other experiments. The regular bursts of activity at approximately 90 minute intervals are clearly evident following onset of major activity.

"Average level of activity" presented in Tables IV and V is expressed as counts per hour since differences in lengths of the activity period precluded direct comparison of active periods.

Attempts were made to average the daily activity records referenced to time of entry into torpor and other arbitrary phase markers. In these attempts the bursts of activity tended to average out and meaningful patterns were not apparent.

Analysis of activity data collected during 30 hours of space flight showed good correlation with body temperature data, but the short record precluded significant conclusions regarding patterns of activity. The implication is that had the nominal mission been completed, the activity data collected would have been useful.

TABLE II

Comparison of the free-running circadian period (τ) of body temperature for pocket mice selected for Experiment S-071. Estimates of τ were derived from times of arousal from torpor. All data were collected in constant dark conditions at an ambient temperature of 20 ± 0.5°C.

	Screening[1]		Pre-Launch Selection[2]		CPE 1[3]	(Control)
Animal Number	τ ± C.I.[5] (Hours)	Dispersion[6] (Hours)	τ ± C.I. (Hours)	Dispersion (Hours)	τ ± C.I. (Hours)	Dispersion (Hours)
2984 M	22.84 ± 0.15	2.09	23.35 ± 0.03	0.39	22.88 ± 0.07	1.54
3291 M	23.50 ± 0.08	0.67	23.59 ± 0.05	0.67	23.46 ± 0.03	0.66
3061 M	23.52 ± 0.08	0.53	23.22 ± 0.03	0.37	22.25 ± 0.07	0.36
2909 M	23.81 ± 0.03	0.34	23.71 ± 0.08	1.02	23.91 ± 0.02	0.31
3375 M	23.31 ± 0.14	1.21	23.83 ± 0.04	0.61	23.04 ± 0.03	0.77
3264 F	23.45 ± 0.06	0.58	23.39 ± 0.07	0.87	22.72 ± 0.09	1.72
					CPE 2[4]	(Flight)
3301 M	23.41 ± 0.06	0.64	23.41 ± 0.05	0.76	23.18 ± 0.26	0.32
3081 M	23.30 ± 0.08	0.80	23.45 ± 0.05	0.82	22.73 ± 0.36	0.44
3337 M	22.47 ± 0.16	2.07	23.52 ± 0.05	0.45	22.82 ± 0.69	0.84
3295 F	22.55 ± 0.08	0.98	23.47 ± 0.11	1.48	22.83 ± 0.35	0.27
3341 M	24.87 ± 0.45	1.03	24.31 ± 0.05	0.68	24.83 ± 0.52	0.64
3307 M	23.85 ± 0.06	0.74	23.82 ± 0.06	0.73	23.20 ± 0.27	0.21

(1) 21 day study in HU/LMU - Fall 1972 (NRTC).
(2) 20 day study in HU/LMU - Summer 1973 (KSC).
(3) 28 day study in flight hardware - Summer 1973 (KSC).
(4) 7 day study in flight hardware - Summer 1973 (KSC).
(5) Estimated free-running period ± 95% confidence interval.
(6) Standard deviation of times of arousal about trend line.

TABLE III

Comparison of the free-running circadian period (τ) of body temperature for pocket mice maintained as reserves in support of Experiment S-071. Estimates of τ were derived from times of arousal from torpor. All data were collected from the HU/LMU under conditions of constant dark at an ambient temperature of $20 \pm 0.5^{\circ}C$.

	Screening[1]		Pre-Launch Selection[2]		Post-Launch[3]	
Animal Number	$\tau \pm$ C.I.[4] (Hours)	Dispersion[5] (Hours)	$\tau \pm$ C.I. (Hours)	Dispersion (Hours)	$\tau \pm$ C.I. (Hours)	Dispersion (Hours)
3320 M	22.87 ± 0.06	0.69	24.03 ± 0.07	0.64	23.71 ± 0.03	0.35
3339 F	23.45 ± 0.04	0.36	23.87 ± 0.06	0.83	24.13 ± 0.04	0.78
2960 F	23.23 ± 0.11	1.51	23.68 ± 0.12	1.05	23.25 ± 0.09	0.92
3261 F	23.91 ± 0.09	0.72	23.59 ± 0.07	0.95	23.70 ± 0.21	1.32
2979 M	22.39 ± 0.14	1.23	23.28 ± 0.09	0.77	22.09 ± 0.09	1.98
2926 M	23.12 ± 0.05	0.60	23.48 ± 0.30	0.74	23.33 ± 0.07	0.55
3351 M	22.82 ± 0.10	0.81	23.74 ± 0.06	0.56	22.53 ± 0.01	1.87
3369 M	23.47 ± 0.10	0.79	23.68 ± 0.06	0.73	23.36 ± 0.02	0.34
3352 M	24.68 ± 0.08	0.53	23.96 ± 0.09	1.05	23.98 ± 0.04	0.78
3405 M	23.94 ± 0.04	0.39	23.79 ± 0.10	0.75	23.33 ± 0.09	1.35
3084 F	23.69 ± 0.04	0.61	23.59 ± 0.07	0.92	23.05 ± 0.07	1.00

(1) 21 day study - Fall 1972 (NRTC).
(2) 20 day study - Summer 1972 (KSC).
(3) 28 day study - Summer 1972 (KSC).
(4) Estimated free-running period ± 95% confidence interval.
(5) Standard deviation of times of arousal around the trend line.

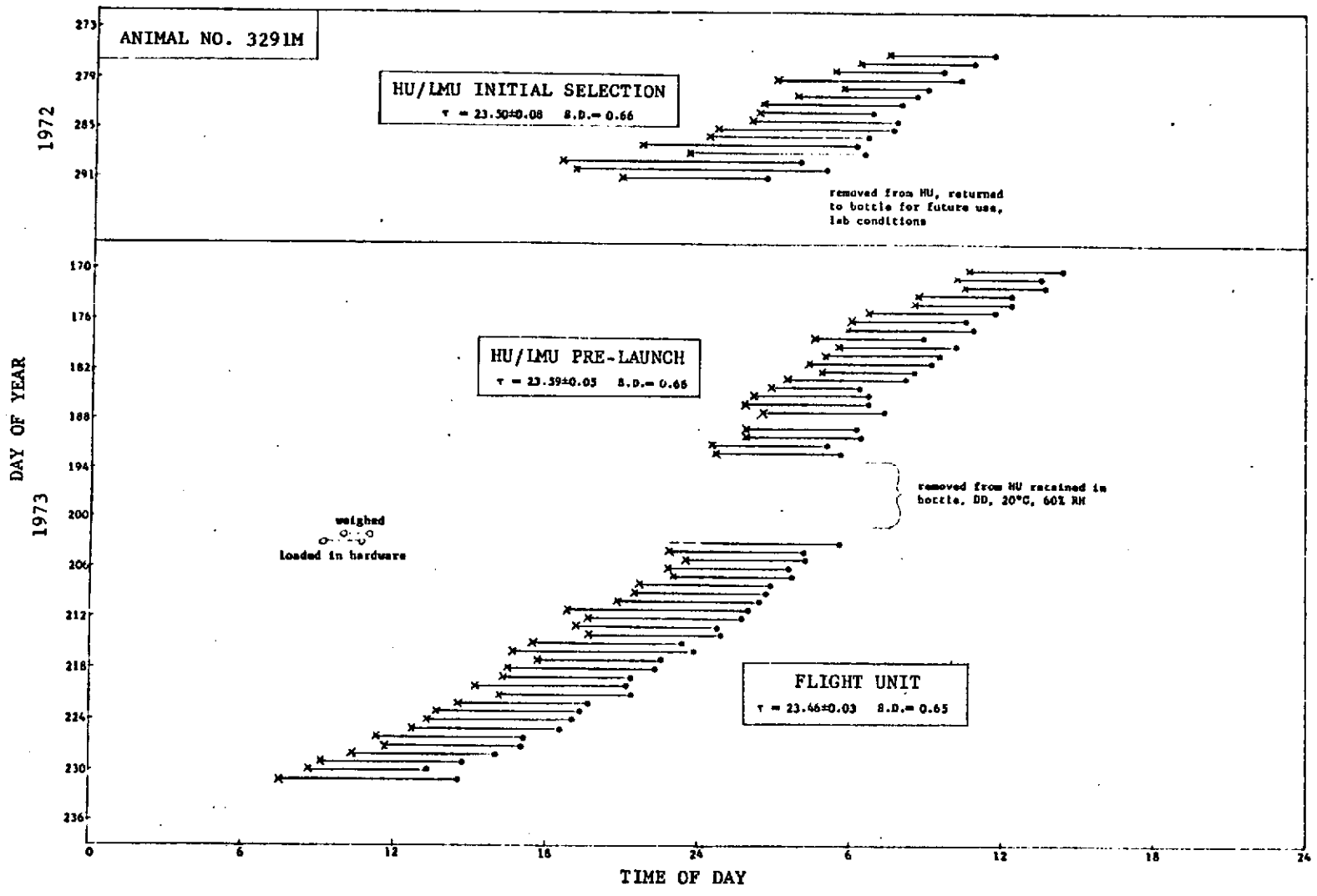

Figure 9. A history of torpid behavior and animal handling for an individual pocket mouse selected for Experiment S-071. Solid bars indicate time in torpor. Free-running period (τ) is estimated from the slope of the curve, fit by the method of least squares, to times of arousal from torpor. Note the similarities in τ values in spite of being measured at different times and in two different kinds of hardware.

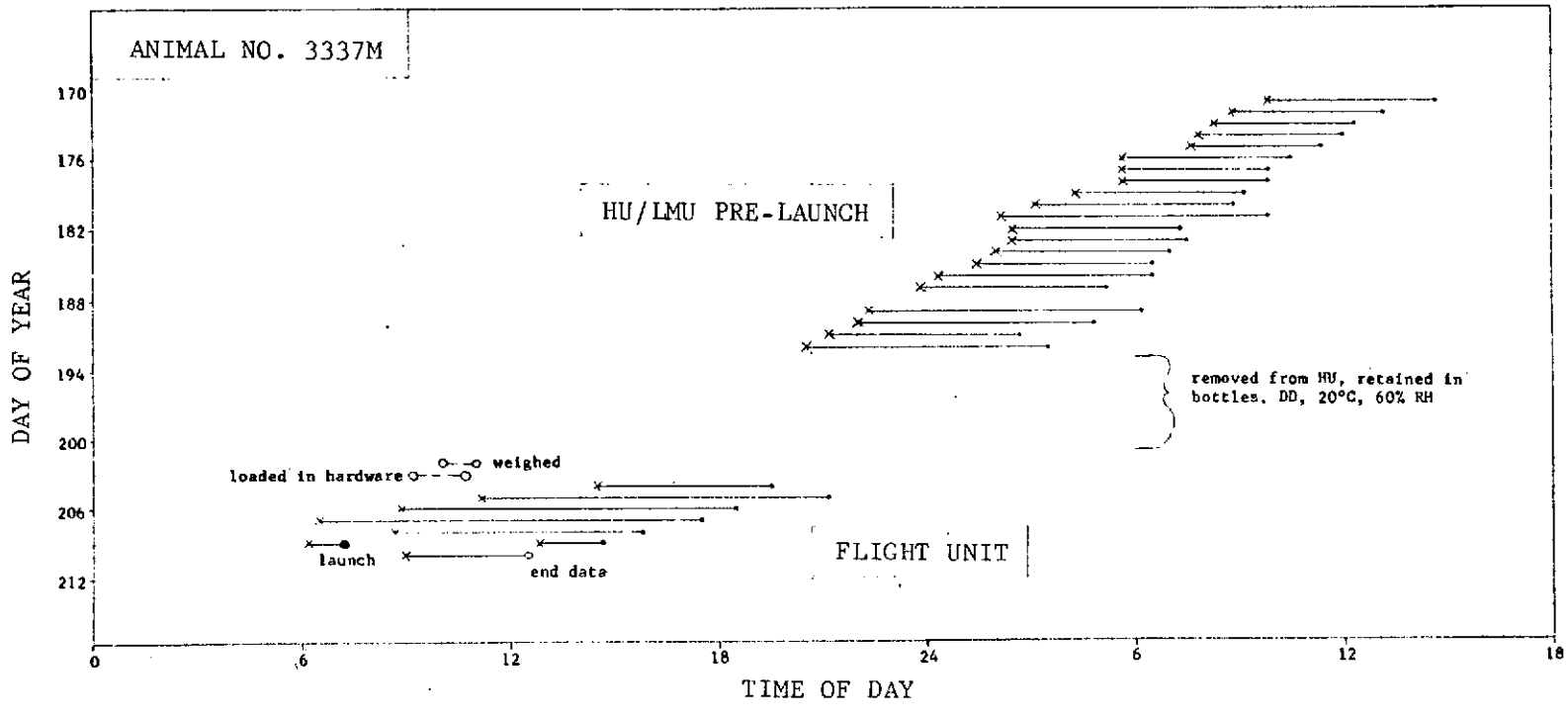

Figure 10. A history of torpid behavior and animal handling of an individual animal from the time of its arrival at NASA/KSC until the demise of Experiment S-071 in space. Solid bars indicate time in torpor. Values for τ are given in Table II.

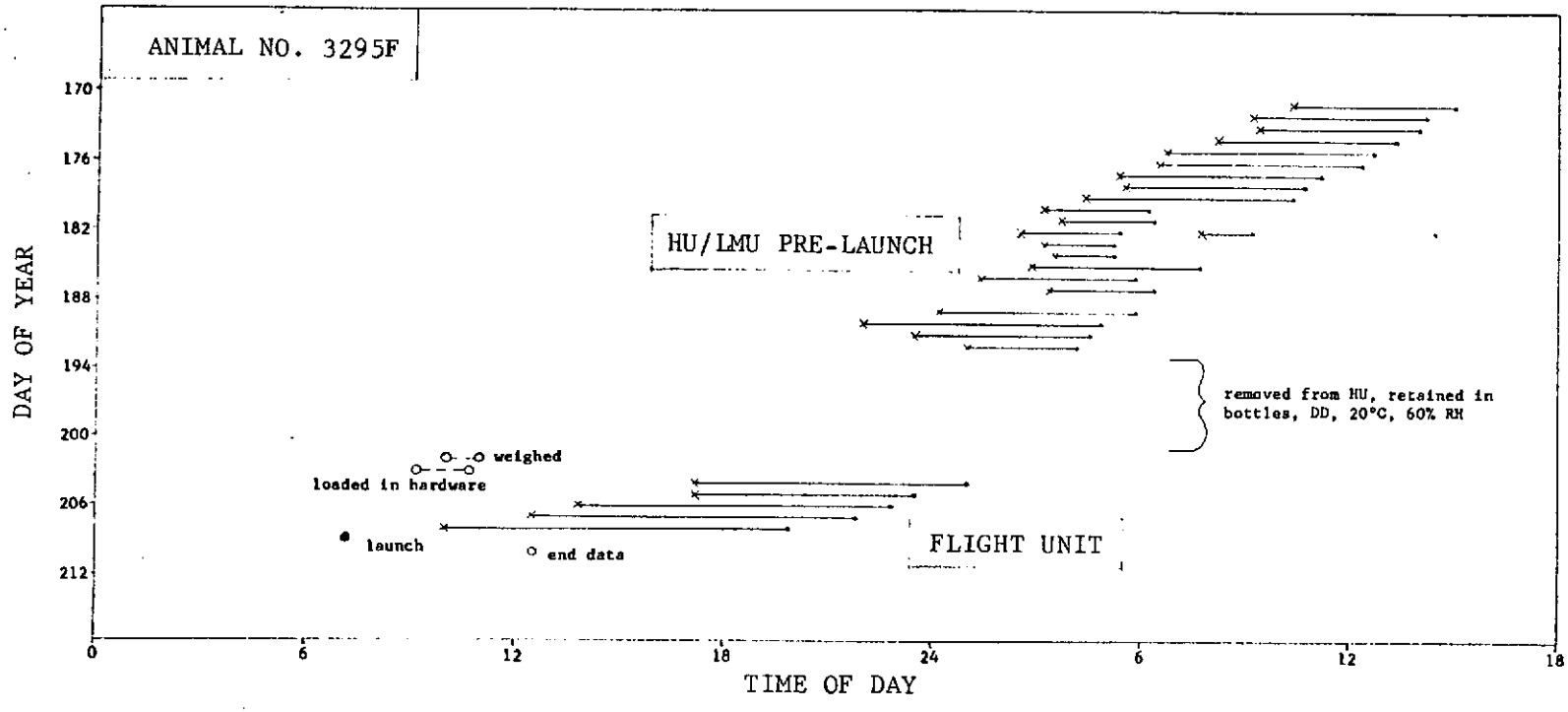

Figure 11. A history of torpid behavior and animal handling of an individual animal from the time of its arrival at NASA/KSC until the demise of Experiment S-071 in space. Solid bars indicate time in torpor. Values for τ are given in Table II.

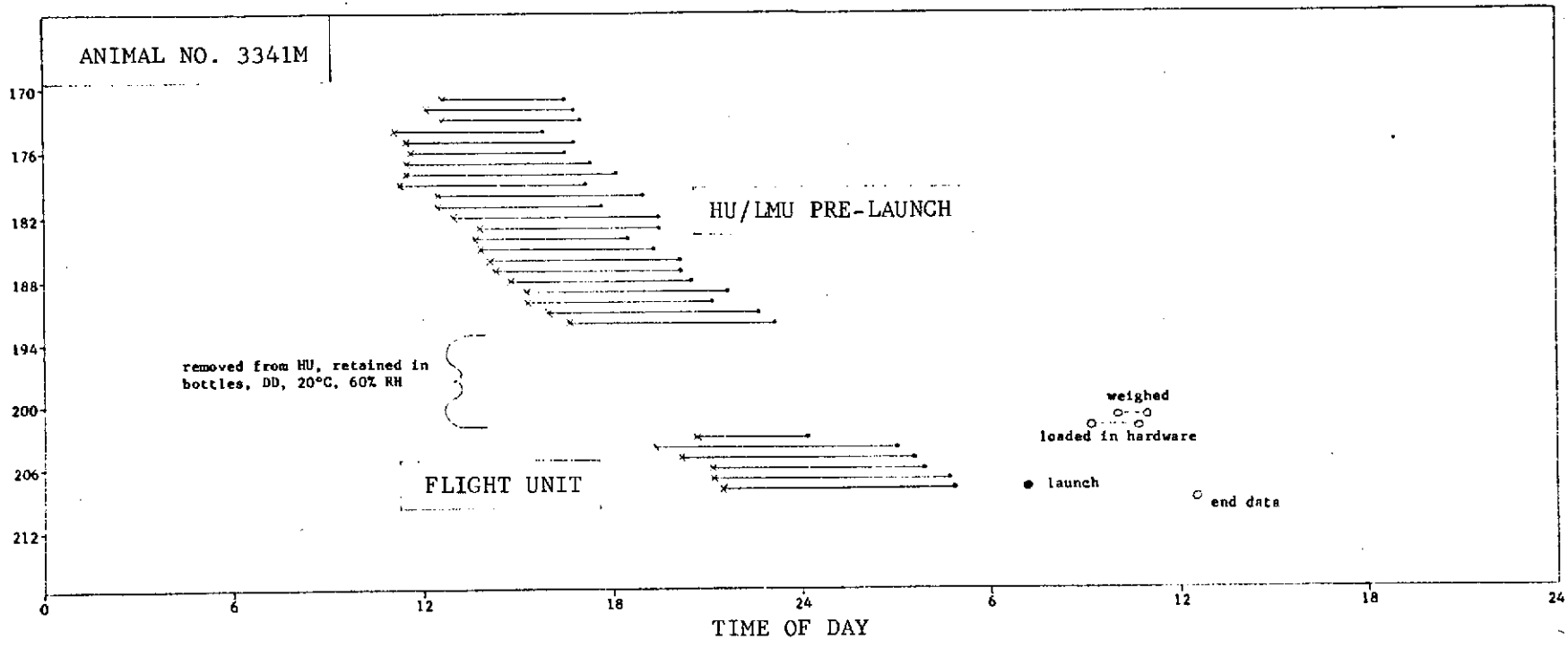

Figure 12. A history of torpid behavior and animal handling of an individual animal from the time of its arrival at NASA/KSC until the demise of Experiment S-071 in space. Solid bars indicate time in torpor. Values for τ are given in Table II.

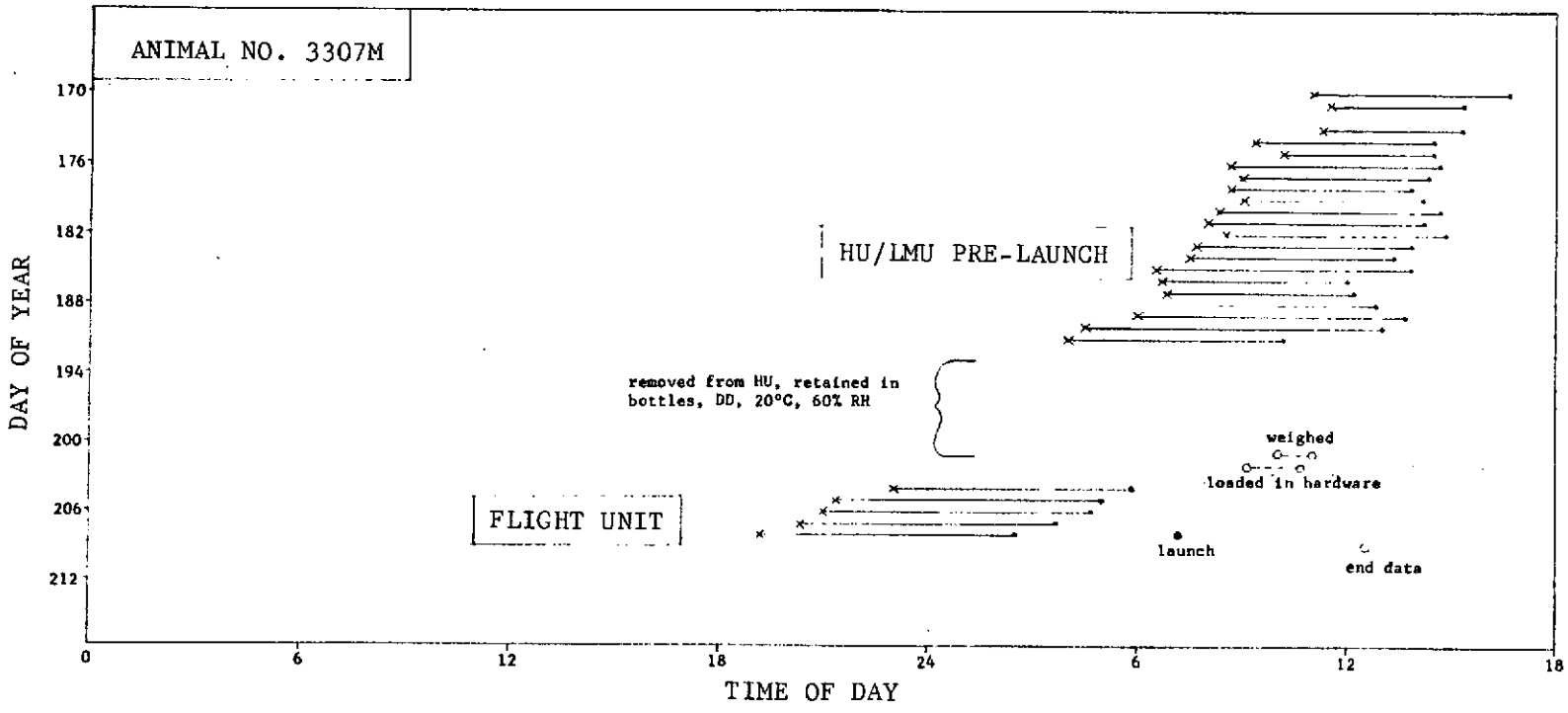

Figure 13. A history of torpid behavior and animal handling of an individual animal from the time of its arrival at NASA/KSC until the demise of Experiment S-071 in space. Solid bars indicate time in torpor. Values for τ are given in Table II.

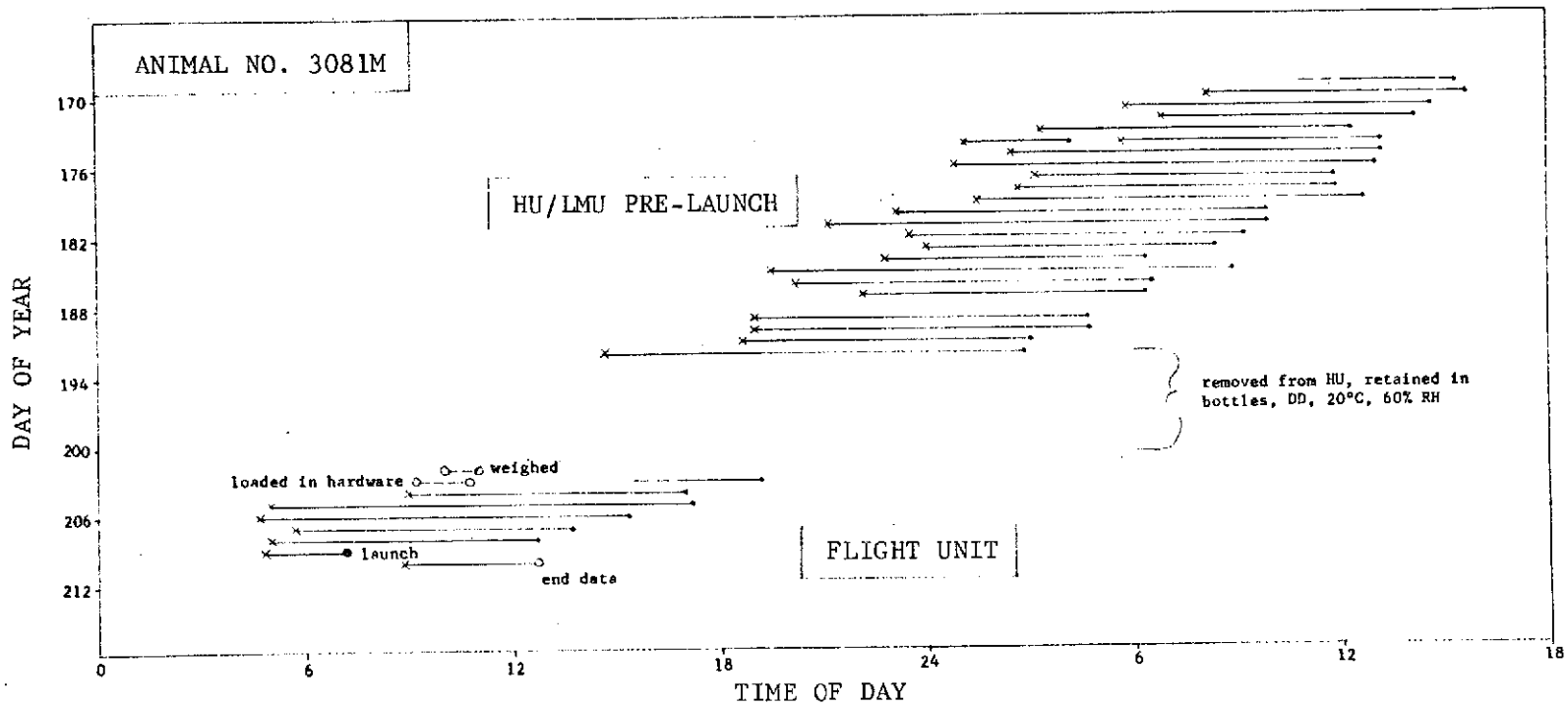

Figure 14. A history of torpid behavior and animal handling of an individual animal from the time of its arrival at NASA/KSC until the demise of Experiment S-071 in space. Solid bars indicate time in torpor. Values for τ are given in Table II.

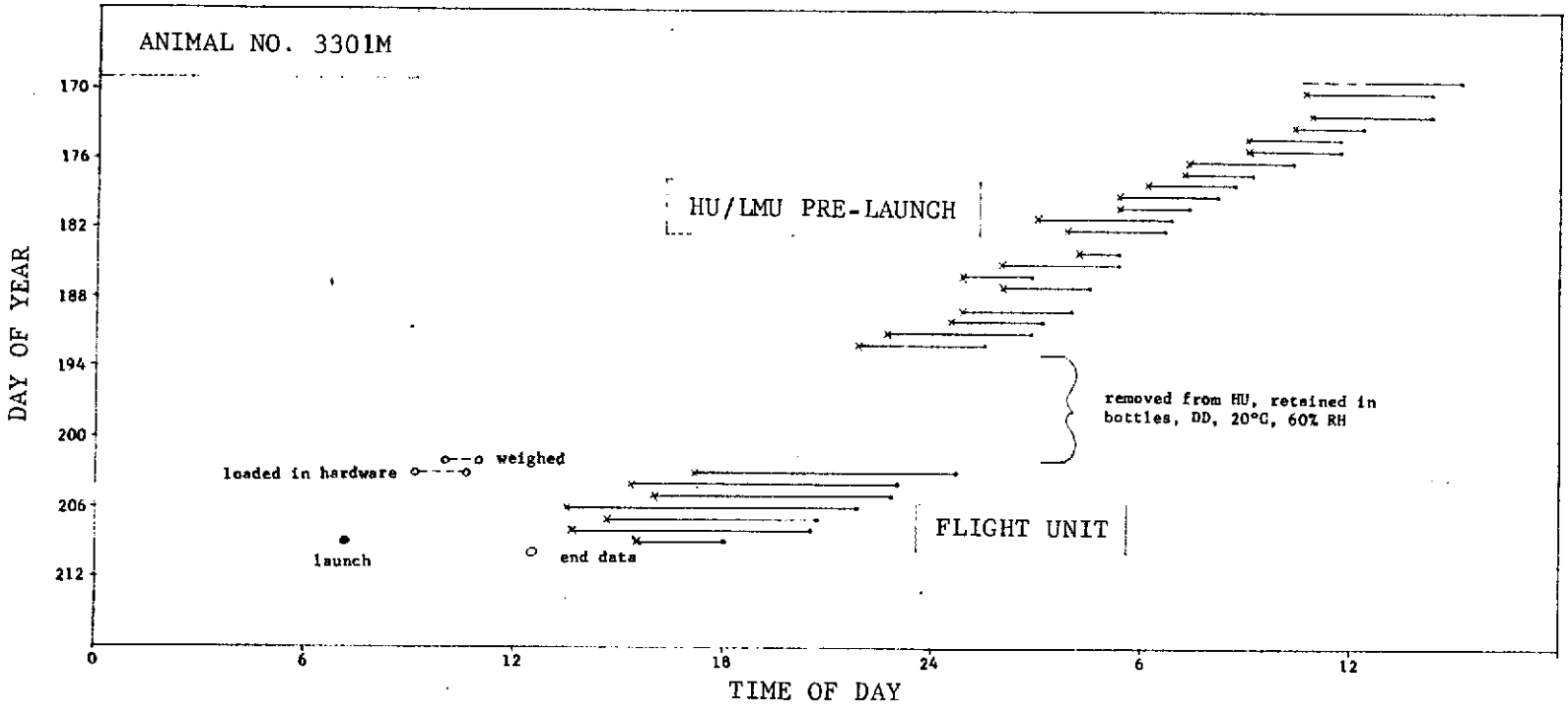

Figure 15. A history of torpid behavior and animal handling of an individual animal from the time of its arrival at NASA/KSC until the demise of Experiment S-071 in space. Solid Bars indicate time in torpor. Values for τ are given in Table II.

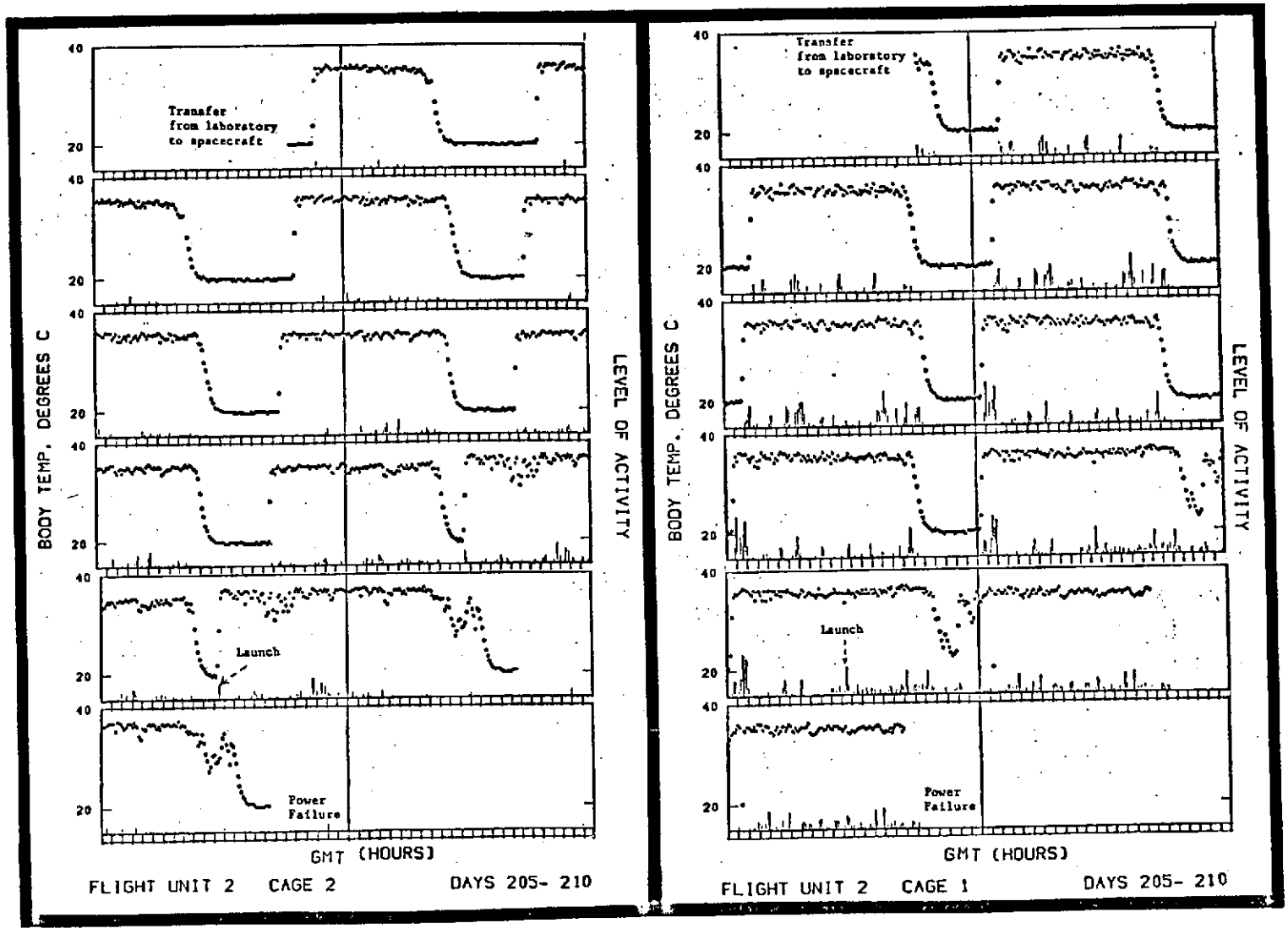

Figure 16. Computer plots of body temperature and animal activity obtained from two pocket mice launched on SL-3. Data are double plotted. Dotted line is body temperature, histogram below is relative activity.

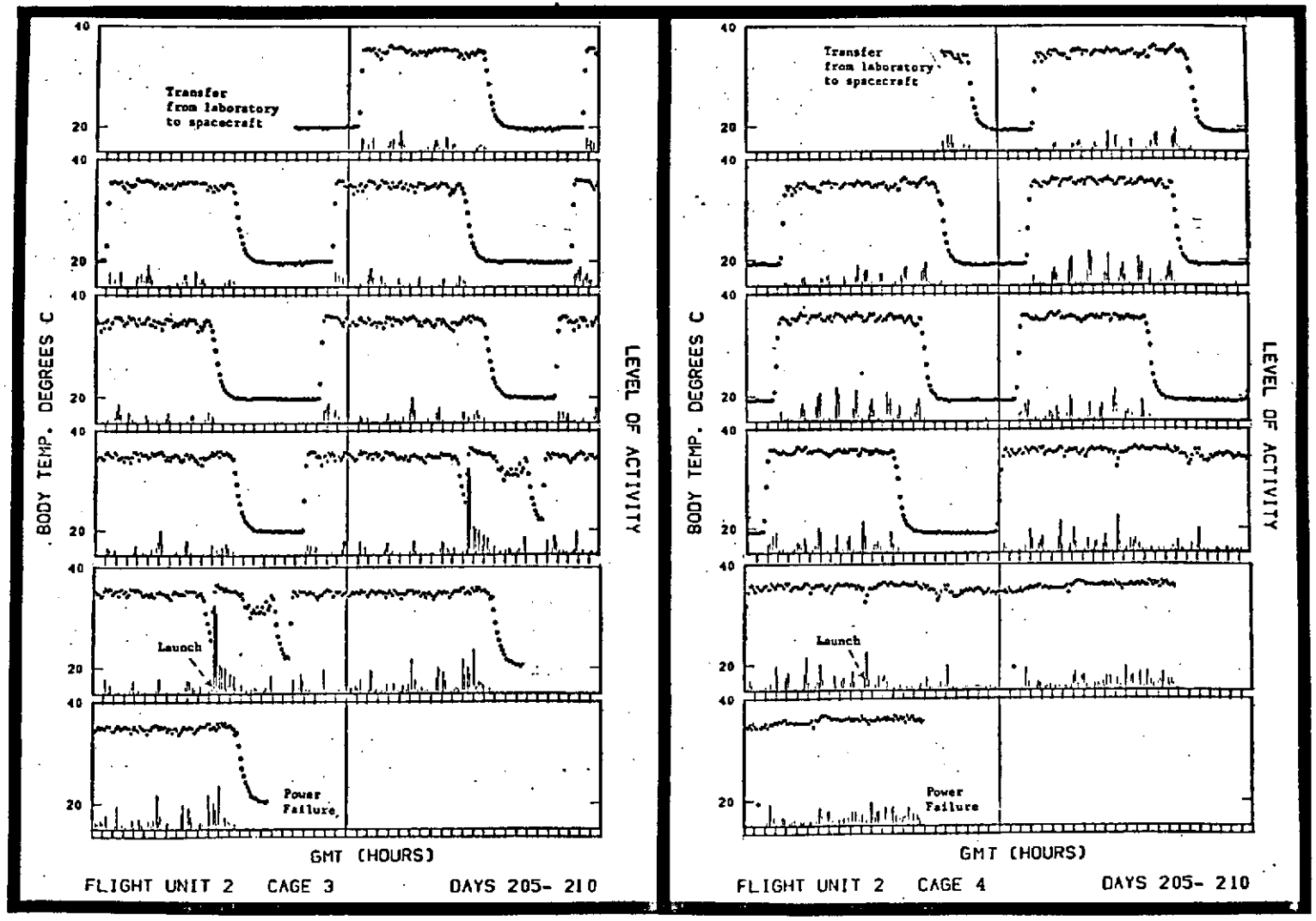

Figure 17. Computer plots of body temperature and animal activity obtained from two pocket mice launched on SL-3. Data are double plotted. Dotted line is body temperature, histogram below is relative activity.

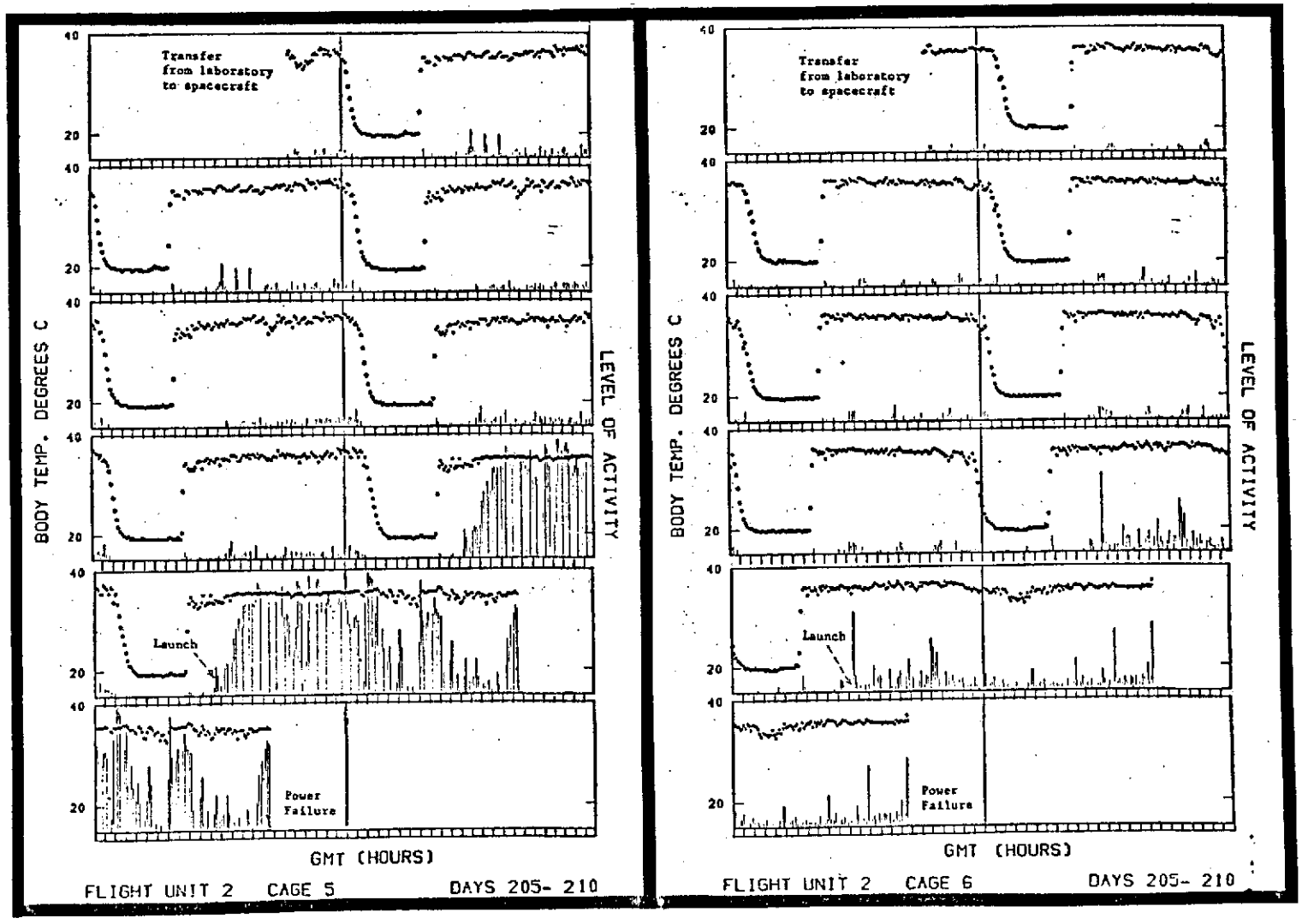

Figure 18. Computer plots of body temperature and animal activity obtained from two pocket mice launched on SL-3. Data are double plotted. Dotted line is body temperature, histogram below is relative activity.

TABLE IV

Characteristics of Activity Expressed by Six Pocket Mice
Housed in S-071 Ground Control Experiment Hardware (CPE 1)
During a 21 Day Period

Cage Number	Animal Sex	Average Activity Level Counts/Hr.	Range	Average Onset of Major Activity (Hrs. Post Arousal)	Estimated Period of Activity Bursts (Minutes)
1	M	77	42-121	4.3	70
2	M	115	99-136	5.2	90
3	M	100	70-135	4.8	75
4	M	101	87-123	4.6	100
5	M	54	24-82	5.0	95
6	F	82	23-127	5.3	110
Mean		88 counts/hr.		4.9 hrs.	90 min.

Table V

Characteristics of Activity Expressed by Six Pocket Mice
Housed in S-071 Flight Hardware (CPE 2)
During the 4 Days Immediately Preceding Launch

Cage Number	Animal Sex	Average Activity Level Counts/Hr.	Range	Average Onset of Major Activity (Hrs. Post Arousal)	Estimated Period of Activity Bursts (Minutes)
1	M	80	54-101	4.1	75
2	M	38	30-44	5.6	60
3	M	74	70-87	3.6	60
4	F	112	88-126	3.8	90
5	M	82	74-86	5.0	80
6	M	57	51-62	4.0	75
Mean		74 counts/hr.		4.3 hrs.	73 min.

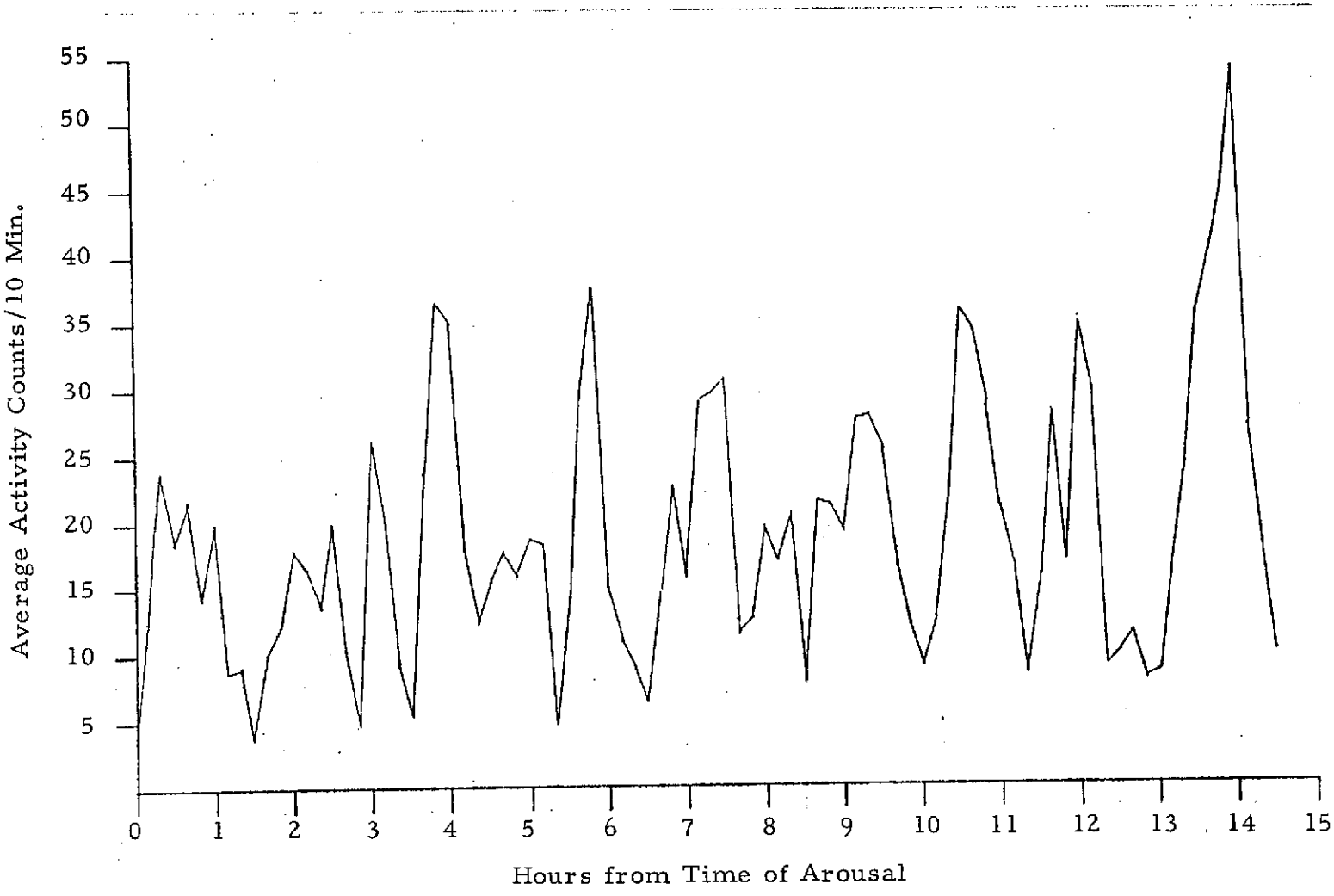

Figure 19. Average daily activity of the pocket mouse in Cage 4 of S-071 space experiment hardware. Data were collected on the four days immediately preceding launch (Days 206-209).

V. DISCUSSION

In a sense, the information presented in this section is a summary of efforts to implement a good intention. The space experiment hardware failed approximately 30 hours after launch. The biological data retrieved during that 30 hours indicated that had the failure not occurred the objectives of the experiment would have been achieved. A question that now arises is whether the experiment can be undertaken on later space missions. From the viewpoint of flight operations, flight qualified hardware is available with all appropriate documentation. Only minor changes are anticipated to avoid the component failure experienced in CPE 2. Experiment support hardware (HU/LMU) is available to serve as a ground control, and data have been obtained to attest to its adequacy as a ground control. If the experiment is not undertaken again a sizable investment, not entirely monetary, will be lost.

The real question is whether the study of circadian organization is still relevant to current NASA missions. In our judgement the answer is an emphatic yes. The scientific literature is replete with evidence of the importance of the stability the temporal organization of life processes to normal efficient functioning of plants, animals, and man. Manifestations of this temporal organization typically are expressed as cyclic phenomenon varying from spontaneous firing of neurons to annual cycles of breeding and migration. Of these cyclic phenomenon those with a period of about 24 hours (circadian) are the most common.

It is something of a paradox that circadian organization is ubiquitous, appears to have real survival value in nature, and if upset can produce undesirable effects. Yet individuals within a normally rhythmic species can be arhythmic in constant environments, and some lower forms of life are typically arhythmic. It follows that circadian organization while characteristic of most eukaryotic forms of life is not in itself essential to survival. It must be noted, however, that even individuals normally arhythmic in constant environments can be entrained (become rhythmic) to

cyclic environmental cues, and in organisms demonstrating circadian organization in constant environments, the disruption of the circadian system reduces the effectiveness of the organism. However one chooses to interpret the data even the most conservative investigators tend to accept a stable circadian system as characteristic of higher forms of life and a measure of physiological well being or homeostasis.

The early literature on circadian periodicity is dominated by demonstration of the ubiquitous nature of the phenomenon. A major milestone was the Bünning Hypothesis which presented an explanation of the way in which cyclic environmental stimuli (zeitgebers) entrain organisms. This contribution was closely followed by the work of Kramer and Von Frisch on birds and bees respectively which demonstrated that organisms did not passively respond to environmental stimuli but relied on their circadian "clock" to mediate behavioral responses. The question of whether cyclic environmental cues actually drove the "biological clock" (exogenous), or simply modified an inherent physiological periodicity (endogenous) was seriously studied through the 1960's. It was this latter question which stimulated submission of a proposal in 1966 to NASA (MSFEB Experiment S-071) to study the stability of the circadian system of pocket mice in space. The question is still pertinent although the justification for the experiment has changed.

In the past 15 years the accumulation of data overwhelmingly supports the premise that circadian organization is indeed endogenous. We no longer believe that the question of the endogenous vs. exogenous nature of circadian periodicity a significant enough question to warrant the high cost of a space biology experiment. However, we are convinced that knowledge of the way in which circadian systems respond to weightlessness is a significant question which relates both to manned space flight, and to the design of future space biology experiments.

It has been argued that data from animal experiments, and pocket mice in particular, cannot be applied directly to man and that if indeed the real concern is human well being the experiments should be done on humans.

We cannot disagree with that argument. Extrapolation of data from one species to another is not only difficult but also dangerous. Animal experiments, even on sub-human primates, can only provide a basis for the calculated risk of human application. It is evident from astronaut performance during the Skylab Program that humans can survive well in weightlessness for at least three months. It is equally evident however that physiological adaptations do occur and that performance while good left room for improvement. The many variables associated with the Skylab missions preclude analysis of possible effects of desynchronosis. In our view disruption of the circadian system in healthy humans is not likely to effect survival but it may be implicated both in rates of adaptation to weightlessness and performance of discriminating tasks.

We would hope that during the Space Shuttle Program sophisticated experiments on human circadian systems will be undertaken. Since human experiments are both complicated and costly it is reasonable to look for some justification for execution derived from hard data. The only evidence to date of weightlessness possibly effecting a circadian system was collected from the Rhesus monkey flown on Biosatellite III. Those data however are ambiguous and additional animal experiments are called for. Since circadian organization is a physiological state characteristic of most forms of life and is not unique to man or mouse, data from animal experiments can provide the needed guidelines for future studies of man in space.

With regard to space biology the sophisticated experimental biologist is acutely aware of circadian phenomenon and his experiments are carefully designed to collect data at comparable times of day. In the process of translating the laboratory experiment to a space experiment it may be forgotten that interpretation of data may be predicated on the unsupported assumption of a stable circadian system in weightlessness. It has been shown that an organism's circadian system can be disrupted by environmental extremes, and that such disruptions can be detrimental. It follows that the behavior of the circadian system in the environmental extreme of weightlessness must be studied.

It is our conclusion that research on factors influencing the stability of circadian organization is not only within the NASA charter but approaches a mandate for guiding development of space biology experiments.

VI. SUMMARY AND CONCLUSIONS

The biology of pocket mice (Genus: Perognathus) has been under study at Northrop for a number of years (Appendix C). The effort supported by both in-house and contract funds has been a mixture of fundamental research directed toward an understanding of the physiology and radiobiology of the Heteromyidae; and applied research directed toward development of space biology experiment hardware. Based on this experience a joint proposal to "Study Circadian Periodicity of Pocket Mice in Space" by Professor C. S. Pittendrigh now at Stanford University and R. G. Lindberg of Northrop Research and Technology Center was submitted to NASA on 5 May 1966, and accepted for initial implementation in near-earth orbit as a part of the Apollo Applications Program. The experiment carried the designation of MSFEB Science Experiment S-071 and was later assigned to the Skylab SL-3 mission.

A Laboratory Test Model of equipment proposed for execution of experiment S-071 was designed and fabricated under Contract NAS2-5093, and tested with instrumented animals. Operation of the Laboratory Test Model verified the hardware configuration and led to selection of the particular species of pocket mouse to be used in the experiment (Perognathus longimembris), and the biological parameters to be measured (body temperature and animal movement). Based on this endorsement, flight hardware was designed and fabricated by the Northrop Electronics Division (Contract NAS2-5850). As a part of flight hardware development, an Animal Holding Unit and a Laboratory Monitoring Unit (HU/LMU) were fabricated and delivered in March 1971 to NCL for operation and maintenance. The HU/LMU has been used to support qualification tests of flight hardware, research on properties of circadian periodicity in pocket mice contained in hardware simulating space flight units,

screening of potentially useful flight animals, and launch site operations connected with execution of Experiment S-071.

In order to define a suitable experiment protocol, laboratory research was directed to solution of the problem of how to condition animals prior to their insertion in the flight hardware. Studies focused on the phenomenon of "after effects" following various photoperiodic regimes (see Part III). A fundamental aspect of the study was the establishment of phase response curves for free-running pocket mice exposed to either a single light stimulus or temperature stimulus at various points in the circadian cycle. These studies, coupled with documentation of the stability of the free-running period over several months' time, indicated that the mice should be exposed to constant dark prior to, and during insertion in flight hardware.

In other exploratory experiments, studies were conducted on various factors which might perturb Experiment S-071 in the course of normal flight operations (see Part II). These factors included cage size and geometry, very low humidity, air velocity through the cage, atmospheric pressure, noise and ambient temperature variations. Of the factors studied, only variations in ambient temperature was judged significant in perturbing the precision of the free running period or causing complicating phase shifts. If the flight hardware operated within specifications, a successful experiment could be expected.

The objectives of Skylab Experiment S-071 were

A. To study the stability of the circadian rhythm of body temperature as reflected in the persistence and precision of the "free-running" period,

B. To similarly study the stability of the circadian period of animal activity, and

C. To look for evidence of changes in phase relationship between the circadian rhythms of body temperature and animal activity as an indication of the stability of the circadian system under conditions of weightlessness.

Had the space experiment continued to operate, we have no reservations but that the objectives of Experiment S-071 would have been realized. Even with the failure, we have every confidence that the remaining space experiment hardware can be used to execute Experiment S-071 on another mission. The experience has demonstrated that:

A. The animals were not overly perturbed by launch stresses, suggesting that meaningful data might be obtained on short missions,

B. The environment experienced by the mice in the HU/LMU is very comparable to that experienced in the flight hardware, at least to the extent that any undocumented variations that may exist do not affect the endpoints being measured. This indicates that the HU/LMU could be used as a ground control for a flight experiments,

C. The animal-telemeter preparation was reliable and may exceed specifications for operating life,

D. The experiment protocol does not introduce complicating stresses on the mice.

Since the scientific rationale for the experiment is still valid, and since no equivalent experiment has been done, it can be concluded that a new flight opportunity should be pursued.

ACKNOWLEDGEMENT

Experiment S-071/S-072 hardware was designed, fabricated, and spaceflight qualified by the Northrop Electronics Division, Palos Verdes, California under NASA contract NAS2-5850 to Ames Research Center. We are grateful to the personnel of both organizations for their efforts to insure the biological compatibility of the experiment hardware.

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PART II

Stability of the "Free-Running" Circadian Period of Body Temperature in Perognathus longimembris Exposed to Simulated Launch and Space Flight Stresses

I. INTRODUCTION

A prime tenet for space biology experimentation is that the phenomenon to be studied in space must be so well documented on the ground that anomalies which may arise in space will be both recognized and properly interpreted. Pocket mice have been under study in our laboratory for a number of years. Research has been directed toward an understanding of the biology, physiology, and radiobiology of the Heteromyidae as well as applied research directed toward development of biologically compatible experiment hardware. (See Appendix C.)

In the context of Experiment S-071, we were concerned with three kinds of problems which could influence the quality of data. The first class of problems were associated with single event perturbations such as the noise, vibration and accelerations associated with launch, or single perturbations in space due to short term loss of temperature control or intermittent accelerations due to spacecraft maneuvers. If these kinds of stimuli, either singly or in combination, are strong entraining agents, then we could anticipate a phase shift but presumably with little effects on the precision of the subsequent free-running circadian period of body temperature (τ_{FR}).

The second class of problem was associated with weak entraining agents that might be experienced during the course of the experiment in space. We could only speculate as to what these might be: perhaps regular firing of solenoids immediately adjacent to the experiment hardware or cyclic fluctuations in temperature due to failure of thermal control, or even periodic changes in acceleration due to astronaut work cycles or spacecraft maneuvers.

The third class of problem related to the well-being of the animal. If injured during launch, or unable to feed in weightlessness, for example, the quality of the data would be compromised. As a consequence, a series of pilot or exploratory experiments were undertaken relating both to design of experiment hardware and operational conditions anticipated to exist for

Experiment S-071. The purpose of these experiments was to identify possible compromising factors for further study.

II. MODIFICATION OF THE "FREE RUNNING" CIRCADIAN PERIOD OF BODY TEMPERATURE (τ_{FR})

A. Effect of Handling and Cage Configuration

Six Perognathus longimembris surviving a continuous six month study of the stability of the circadian period (τ_{FR}) were removed from their cages under red light and placed in scintered polyethylene tubes (6.5 cm diameter x 20.3 cm long). After equivalent degrees of agitation (about 5 minutes of slow rotation and tumbling in the tubes), three animals were returned to the normal cage and three, confined to their tubes with adequate food, were placed inside the regular cage in constant darkness. Monitoring of body temperature was reinitiated and continued until the end of the study. One telemeter failed in the course of the experiment. Data from the five remaining animals is summarized in Table VI.

The disturbance apparently had no effect on the expression of the free-running circadian period of body temperature. No phase shifts were observed.

The Laboratory Test Model (LTM) of experiment hardware was used to conduct two experiments with Perognathus longimembris. In one case a cage height of 6.5 cm was used and the cage assembly ventilated with constant temperature air at ~ 50-60% RH. In the second case a 4 cm cage height was used and the cage assembly ventilated with dehumidified air (>5% RH) at constant temperature. The τ_{FR} was comparable in both experiments and no effect of the shallow cage and dry air were observed (Table VII).

B. Effect of Simulated Launch Stress

With regard to the launch environment, pocket mice, implanted with biotelemeters and housed in Experiment S-071 flight hardware, have been exposed to vibrations and accelerations at the qualification levels set for

TABLE VI

Effect of handling and cage configuration on the stability of the free-running circadian rhythm of body temperature in Perognathus longimembris.

ANIMAL	τ_{FR} (Hours ±95% C. I.)	DISPERSION (± Hours)	$\Delta\tau_{FR}$ (Hours)	Δ DISPERSION (Hours)
2477M (a)	24. 1 ± 0. 17	0. 73		
(b)*	24. 0 ± 0. 07	0. 16	- 0. 1	- 0. 57
2474M (a)	22. 8 ± 0. 02	0. 10		
(b)*	23. 1 ± 0. 03	0. 06	+ 0. 3	- 0. 04
2429M (a)	23. 4 ± 0. 02	0. 08		
(b)*	23. 1 ± 0. 15	0. 34	- 0. 3	+ 0. 26
2619F (a)	23. 5 ± 0. 13	0. 29		
(b)	23. 3 ± 0. 06	0. 13	- 0. 2	- 0. 16
2463M (a)	23. 7 ± 0. 01	0. 05		
(b)	23. 7 ± 0. 03	0. 08	-0-	+ 0. 03

(a) τ_{FR} determined from 21 days in standard monitoring cage

(b) τ_{FR} determined from 14 days in standard monitoring cage following handling

(b)* Animals returned in porous polyethylene tubes (4. 4 cm diam. x 20. 3 cm long) for 14 days following handling

the experiment hardware (1). In addition, instrumented mice in cage configurations similar to Experiment S-071 were exposed to the more rigorous vibration, acceleration and acoustic environments simulating launch by a McDonnell Douglas DSY-3E booster with a FW-4 third stage (2). Instrumented mice have also been subjected to simulated vibration and acceleration forces of an Atlas-Agena launch (3). These tests, while limited, produced no casualties and only transient effects on the circadian periods. The nature of the engineering tests, in some cases, necessitated exposing the mice to light just prior to test. Since exposure to light alone could have accounted for the transient effects observed (Part III), we cannot be certain that the launch stresses per se caused any effect. These tests did clearly establish the high tolerance of instrumented free-ranging mice to launch stresses, and the fact that launch stresses are not likely to compromise Experiment S-071.

C. Effect of Chronic Acceleration

Centrifuge studies showed that some mice sensed the starting and stopping of the centrifuge and responded by phase shifting τ_{FR}. There was no evidence from the pilot study to indicate that τ_{FR} was changed or degraded by chronic acceleration to 2.2G (Appendix D). Since accelerations in Skylab were anticipated to be very low, even during attitude changes, accelerations were not anticipated to compromise the experiment.

D. Effect of Noise

Pocket mice have a keen sense of hearing and are disturbed by noise. We have no evidence, however, that noise is an effective entraining agent (4). One mouse exposed to regimens of one hour and four hours of noise (8 dB above ambient) once each day initially was disturbed and responded as though entrained but appeared to accommodate within a few days and proceeded to free-run even though the noise regime was continued (Figure 20). In that other mice did not respond to the noise, it is doubtful that low level inflight noises, should they occur, would introduce anymore than transient effects.

In another experiment six mice housed in LTM hardware were exposed to a single loud noise of 2 hours duration. The noise was produced by arranging a metal clapper to rap once a second on the metal housing. The level of noise was not measured but was loud enough to disturb personnel in adjacent laboratories. This experience failed to perturb the circadian period of the mice.

E. Effect of Varying Partial Pressure of Oxygen

The space experiment hardware was designed as a gas tight enclosure with zero or very low leak rate. Should a large leak occur, the effect would be catastrophic, but a small leak would result in a gradual loss of nitrogen and an enrichment of oxygen. Radiobiological studies have demonstrated the tolerance of P. longimembris to high oxygen tension (5), and pilot experiments with 100% oxygen at reduced pressure indicate that τ_{FR} is unaffected between 120-350 mm Hg. Even higher pressures would probably be ineffective. Should the oxygen make-up regulator malfunction, the mice could be exposed to large cyclic pressure changes. Studies indicated that cyclic exposure to 12 hours at 760 mm Hg and 12 hours at 830 mm Hg entrained only two of four animals suggesting that normal barometric changes are not effective zeitgebers for pocket mice (6). Studies done within the specifications of the experiment hardware (700 ± 15 mm Hg) showed no evidence of perturbing τ_{FR}.

F. Effect of Low Relative Humidity

Relative humidity had no effect on τ_{FR} but animals held at low relative humidity characteristically loose weight (Table VII). Weight loss in both astronauts and monkeys flown in space is generally attributed to water loss (7). If water loss is a normal consequence of weightlessness, the pocket mouse presented a special problem. Since it does not drink water, there would be no way to make up water loss except by metabolizing more food. It is possible that being adapted to an arid environment the pocket

Figure 20. Response of Perognathus longimembris to two different regimens of daily noise. Noise was administered first, for one hour each day and second, for four hours each day (shaded area). Source of noise was the release of compressed air at 30 psi for ~3 sec every 13 sec (277 pulses/hr). A solenoid "snap" was associated with each air burst. Measurement of increased sound level was difficult because of the slow response time of the sound meter which did not respond to either the solenoid "snap" or the initial "hiss" of the air. Maximum readings were only 8 dB above ambient. The increase in noise level was judged low.

Horizontal bars indicate time the mouse was in torpor; solid dots, time of arousal from torpor; and open triangles, arousals from torpor caused by disturbances other than noise.

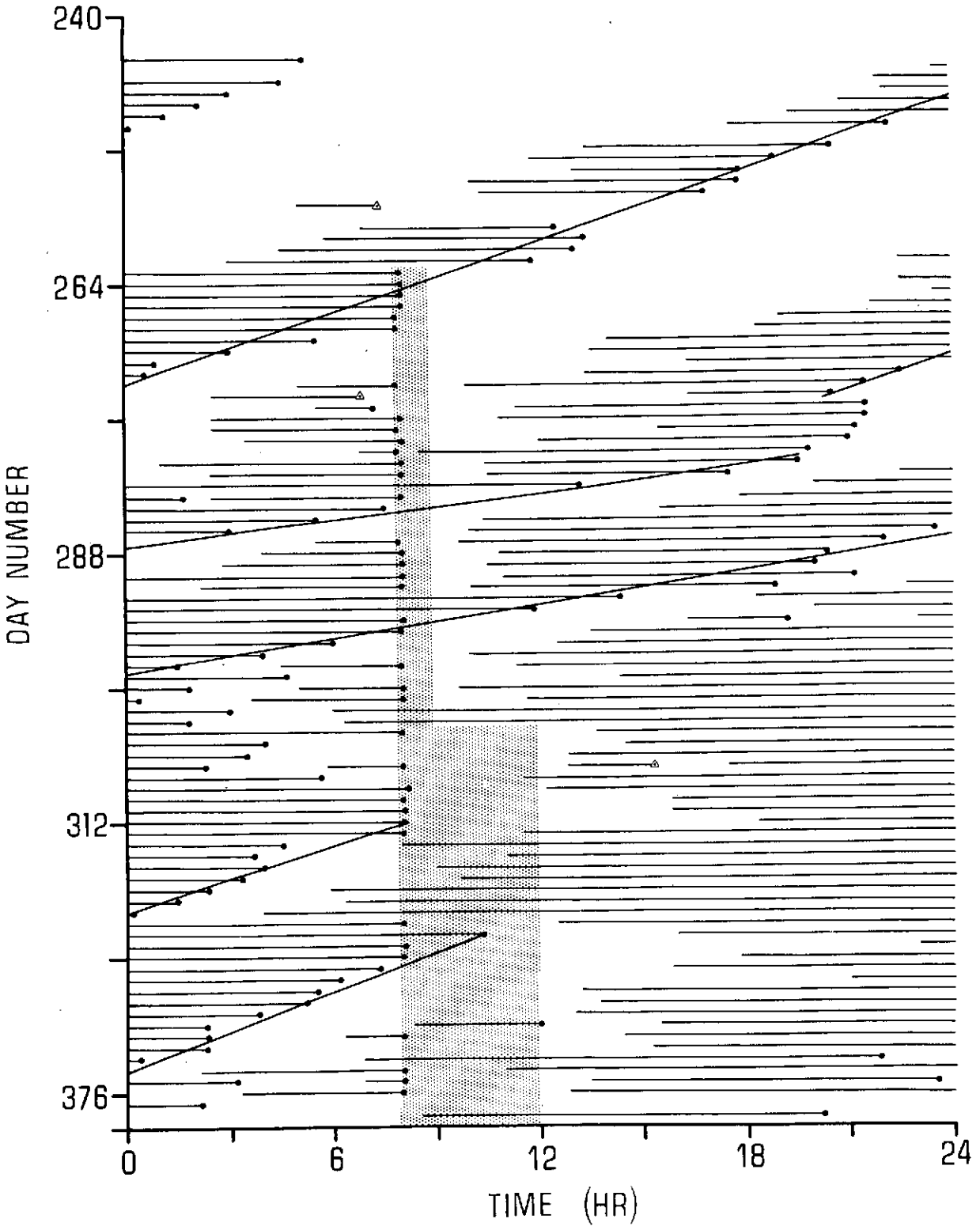
240
264
288
312
376
DAY NUMBER
0
6
12
18
24
TIME (HR)

TABLE VII

Effect of cage height and low relative humidity on the stability of the free-running circadian rhythm of body temperature in Perognathus longimembris held in prototype experiment hardware under conditions of constant dark and constant temperature (± 0.5° C).

ANIMAL	CAGE CONDITIONS	τ (Hours ±95% C.I.)	DISPERSION (± Hours)	$\Delta\ \tau$ (Hours)	Δ DISPERSION (Hours)
2659M	6.5 cm ceiling 50-60% R.H.	22.2 ± 0.02	0.11		
	4.4 cm ceiling < 5% R.H. *	23.6 ± 0.05	0.16	+ 1.4	+ 0.05
	4.4 cm ceiling < 5% R.H. **	23.1 ± 0.05	0.16	- 0.5	-0-
2684F	6.5 cm ceiling 50-60% R.H.	24.9 ± 0.98	3.44 ♣		
	4.4 cm ceiling < 5% R.H. *	23.3 ± 0.06	0.20		
	4.4 cm ceiling < 5% R.H. **	22.7 ± 0.14	0.60	- 0.6	+ 0.4
2675F	6.5 cm ceiling 50-60% R.H.	23.8 ± 0.07	0.26		
	4.4 cm ceiling < 5% R.H. *	23.9 ± 0.04	0.14	+ 0.1	- 0.12
	4.4 cm ceiling < 5% R.H. **	23.8 ± 0.05	0.17	- 0.1	+ 0.03

* First 21 days of regimen.

** Second 21 days of regimen.

♣ Large dispersion due to spontaneous phase shift. Tau on either side of shift ~ 23 + hours.

mouse may be preadapted to that particular effect of weightlessness. The relative humidity in the experiment hardware was maintained at 60 ± 10% RH to minimize evaporative water loss.

G. Effect of Starvation

One possible failure mode of Experiment S-071 was that the mice would be unable or unwilling to feed in a weightless condition. Observation of mice during short term weightlessness induced aboard aircraft were reassuring in that the mice did not appear to panic and should be able to feed if they wish. Five Perognathus longimembris flown on Apollo 17 as subjects for the Biocore experiment did feed under weightless conditions (8). Studies of τ_{FR} in starved animals show that removal of food induces long torpors, tends to improve the precision of τ_{FR} but with a change in the length of τ_{FR} (Figure 21 and Table VIII). It was not clear whether data from starved animals would be useful in meeting the objectives of Experiment S-071.

H. Effect of Changes in Cage Air Temperature

Laboratory experiments with P. longimembris were conducted normally in a constant thermal environment (± 1.0°C). Under these conditions good precision of τ_{FR} has been observed. However, studies recently completed indicate that pocket mice will entrain to 3°C administered for six hours once each day (Part III). Exposure to single event excursions in ambient temperature frequently result in a phase shift, but the subsequent τ_{FR} was stable. The experiment hardware provides a constant ambient temperature of 20° ± 0.5°C. A failure in thermal control could have resulted in loss of the experiment.

III. SUMMARY AND CONCLUSION

A series of pilot experiments relating to both hardware design and operational conditions of Experiment S-071 were undertaken to test the possible effects of various environmental conditions on τ_{FR}.

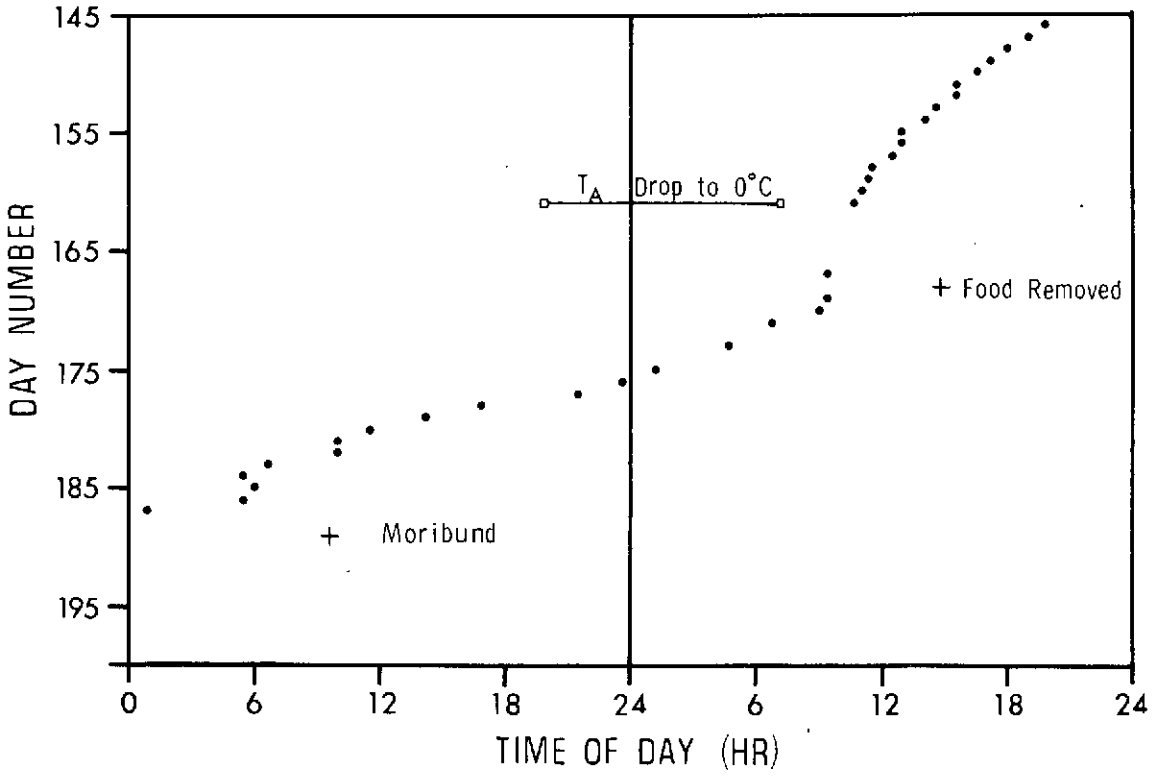

Figure 21. Changes in the free-running circadian period of body temperature in Perognathus longimembris induced by removal of food. Data points are time of arousal from torpor plotted on successive days. Experiment conditions were constant dark and constant temperature ($20^{\circ} \pm 0.5^{\circ}C$) except for one occasion during which failure in the refrigeration system resulted in a lowering of ambient temperature (T_A) to near $0^{\circ}C$ for 12 hours.

TABLE VIII

Effect of Food Removal on τ_{FR} in Perognathus longimembris

Animal Number	τ_{FR} Pre-Food Removal (Hours)	τ_{FR} Post Food Removal (Hours)	$\Delta\tau_{FR}$* (Hours)	Survival (Days)
1	24.4	24.6	+0.2	8
2	23.8	23.0	-0.8	17
3	23.9	22.9	-1.0	6
4	23.9	23.8	-0.1	21
5	23.9	24.3	+0.4	7
6	23.9	22.0	-1.9	6
7	23.4	21.0	-2.4	7
8	23.9	25.3	+1.4	10
9	23.8	20.0	-3.8	6
10	23.7	21.7	-2.0	5

*Seven of 10 mice responded by a decrease in τ by an average of 7.2% (range 0.4 to 16.0%). Three of ten mice responded with an increase in τ by an average of 2.8% (range 0.8 to 5.9%).

Studies were conducted on possible effects produced by cage configuration, animal manipulations, launch accelerations, chronic acceleration, noise, atmospheric composition including changes in pO_2 and relative humidity and pressure, starvation, and changes in air temperature.

None of the factors studied produced effects anticipated to compromise Experiment S-071 if the hardware operated within specifications. Phase shifts were observed to occur following changes in acceleration during centrifuge studies but the subsequent τ_{FR} while different in length did not degrade in precision. Changes in air temperature had significant effects on the precision of τ_{FR} and single temperature excursions could produce pronounced phase shifts. Study of effects of air temperature change were undertaken upon request of engineers who identified loss of precise thermal control of the space experiment hardware as a likely failure mode. The study, reported in detail in Part III, confirmed the need to insure temperature control in the space experiment hardware to within $\pm 0.5^{\circ}C$ of a given set point.

In conclusion, we were confident that Experiment S-071 could be successfully executed in space. We were also confident that enough in-depth knowledge of P. longimembris had been accrued to properly interpret data derived from Experiment S-071.

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PART III

Characteristics of the Circadian Rhythm of Body Temperature in the Little Pocket Mouse, Perognathus longimembris

I. INTRODUCTION

A prime tenet for space biology experimentation is that the phenomenon to be studied in space must be so well documented on the ground that anomalies which may arise in space will be both recognized and properly interpreted. Since the objectives of Experiment S-071 were to measure the persistence and precision of the "free-running" circadian system of pocket mice in space, considerable effort was devoted to the study of that phenomenon on the "ground." Both laboratory monitoring systems and space experiment hardware were used to obtain data.

Additional studies were undertaken to gain better understanding of the circadian system of Perognathus longimembris. Those studies included entrainment of the body temperature rhythm to cycles of light, temperature, and atmospheric pressure, as well as speculations as to the fit of observational data to current models of circadian periodicity.

Radiotelemetry was chosen as the monitoring method and it is possible that decision has bearing on the interpretation of data. A radiotransmitter was implanted in the abdomen of each mouse. There is no evidence that this form of instrumentation in any way affected either behavior or life expectancy apart from the obvious risks of surgery. The technique did necessitate selecting animals of minimum weight (8-10g). In addition animals showing a predilection for torpidity were selected for study. It is estimated that 15-20% of animals collected from the field met these requirements. Therefore, our data are from a select population of animals showing a tendency to express torpor throughout the year.

There is a question as to whether torpor represents a winter physiology, or conversely, whether P. longimembris normally expresses torpor in the field during summer (1). Arguments based on good data have not resolved the question. Data reported herein are derived from a population of animals as nearly alike as can be obtained from multiple field samples collected over a span of several years. If indeed the animals selected

for study represent winter-type animals, then at least all data reported are from winter-type animals.

With regard to characterizing circadian periodicity per se, the data do not show significant differences between animals which express torpor and those that do not (Table IX and X).

II. CHARACTERISTICS OF THE FREE-RUNNING CIRCADIAN PERIOD (τ_{FR}) OF BODY TEMPERATURE

A. Methods

Adult mice were selected for study on the basis of their body weight (8-10g), and their predilection to express torpor. All mice were collected in the field from the area of Palm Springs, California.

A temperature sensing transmitter was implanted in the abdomen of each mouse (2). Individual mice were placed in ventilated plastic cages (10 x 16 x 30 cm) suitably wired to receive the transmitter signal. Body temperature was monitored remotely once every 10 minutes. An excess of air dried seeds was provided ad libitum. Sawdust was provided for bedding. Each cage was placed in an insulated box (~ 60 liters) located in a constant temperature room ($20^{\circ} \pm 0.1^{\circ}C$). Air temperature was lowered to $10^{\circ} \pm 0.1^{\circ}C$ for study of temperature compensation. A small exhaust blower at the air outlet of each box drew air through the box from the constant temperature room.

Infrequent manipulations of the mice, including weighing, feeding, and anesthetizing in preparation for surgery, were done with the aid of a shaded ruby red photographic safelight demonstrated to have no effect on the circadian rhythm of body temperature. The rest of the time the experiments were conducted in constant dark (DD). Either, time of arousal from torpor each day was used as the phase reference point (ϕ_R) of the circadian rhythm for estimating τ_{FR} of body temperature, or autocorrelation of 21 days of continuous data. Estimates based on ϕ_R were preferred since they

represented a clearly identifiable marker of biological origin rather than a statistical derivation. τ_{FR} was estimated by autocorrelation for those cases in which τ_{FR} was compared between mice expressing and not expressing torpor.

B. Results

1. Persistence of τ_{FR}. The free-running circadian period of body temperature was followed in 15 animals for up to seven months (Tables IX and X). The data indicate the presence of a persistent circadian rhythm and, in some cases, remarkable precision. Evaluation of precision was based upon the "dispersion" value which represents the standard deviation of values about the trend line. Autocorrelation tends to smooth the data and dispersion values so derived are invariably smaller than similar values derived from a least squares fit of ϕ_R's. It should be noted that the data are not continuous. Twenty-one days of continuous data out of each month were selected for analysis based upon the availability of data from the monitoring system rather than upon statistical or biological criteria. There appeared to be no difference in the properties of τ_{FR} between males and females. Males expressed torpor in approximately 75% of the sampling periods and females approximately 60%.

In the course of other experiments 11 mice were followed for up to 385 days. The duration of the experiment necessitated replacement of the radiotransmitters. Animals were anesthetized in DD under red light and removed to the laboratory where the old radiotransmitter was surgically replaced. The mouse was returned to DD within 20 minutes while still anesthetized and post surgical recovery occurred within the monitoring system. The effect of this treatment on τ_{FR} is summarized in Table XI. Effects on the length of τ_{FR} ranged from +0.75 hrs to -0.67 hrs. Phase shifts were observed from +1.0 hrs to -3.5 hrs. On the whole, however, surgical insult had slight effect on precision of the subsequent τ_{FR}.

TABLE IX

Free-running circadian period of body temperature in male pocket mice (Perognathus longimembris) maintained in DD at $T_A = 21^{\circ}C \pm 1.5$ for up to 7 months beginning 7 August 1969. Tau was determined by autocorrelation for the periods 3-23 September, 8-26 November, 8-28 January, and 2-23 February.

ANIMAL NUMBER	TORPOR EXPRESSED	MONTH	τ* (Hours)	$\Delta\tau$ (Hours)	DISPERSION** (± Hours)	Δ DISPERSION (Hours)
1	0	Sept.	24.4 ± 0.15		0.52	
	0	Nov.	24.0 ± 0.36	- 0.4	0.83	+ 0.31
	intermittent	Jan.	24.1 ± 0.17	+ 0.1	0.73	- 0.10
	+	Feb.	24.0 ± 0.07	- 0.1	0.16	- 0.57
2	+	Sept.	24.4 ± 0.03		0.14	
	+	Nov.	23.0 ± 0.02	- 0.4	0.07	- 0.07
	+	Jan.	22.8 ± 0.02	- 0.2	0.10	+ 0.03
	+	Feb.	23.1 ± 0.03	+ 0.3	0.06	- 0.04
3	+	Sept.	23.7 ± 0.09		3.76	
	+	Nov.	23.1 ± 0.06	- 0.6	0.17	- 3.69
	+	Jan.	23.4 ± 0.02	+ 0.3	0.02	- 0.15
	+	Feb.	23.1 ± 0.15	- 0.3	0.34	+ 0.32
4	+	Sept.	23.8 ± 0.12		0.52	
	+	Nov.	23.5 ± 0.09	- 0.3	0.26	- 0.26
5	+	Sept.	23.4 ± 0.10		0.43	
	+	Nov.	23.7 ± 0.15	- 0.3	0.44	+ 0.01
6	0	Sept.	23.7 ± 0.04		0.15	
	+	Nov.	23.5 ± 0.13	- 0.2	0.39	+ 0.24
	+	Jan.	23.7 ± 0.01	+ 0.2	0.05	- 0.34
	+	Feb.	23.7 ± 0.03	-0-	0.08	+ 0.03
7	+	Sept.	24.3 ± 0.03		0.12	
	0	Nov.	24.1 ± 0.13	- 0.2	0.30	+ 0.18
	+	Jan.	24.4 ± 0.10	+ 0.3	0.36	+ 0.06

* $\tau \pm$ 95% Confidence Interval

** Standard deviation of plotted values about the trend line

TABLE X

Free-running circadian period of body temperature in female pocket mice (Perognathus longimembris) maintained in DD at $T_A = 21^\circ C \pm 1.5$ for up to 7 months beginning 7 August 1969. Tau was determined by autocorrelation for the periods 3-23 September, 8-26 November, 8-28 January, and 2-23 February.

ANIMAL NUMBER	TORPOR EXPRESSED	MONTH	τ* (Hours)	$\Delta\tau$ (Hours)	DISPERSION** (± Hours)	Δ DISPERSION (Hours)
1	+	Sept.	23.6 ± 0.03		0.12	
	+	Nov.	23.2 ± 0.11	- 0.4	0.32	+ 0.20
2	0	Sept.	23.8 ± 0.09		0.40	
	0	Nov.	23.4 ± 0.19	- 0.4	0.56	+ 0.16
3	+	Sept.	23.4 ± 0.04		0.18	
	+	Nov.	23.5 ± 0.10	+ 0.1	0.29	+ 0.11
	+	Jan.	23.4 ± 0.07	- 0.1	0.31	+ 0.02
4	0	Sept.	23.7 ± 0.12		0.51	
	0	Nov.	23.7 ± 0.16	-0-	0.46	- 0.05
	0	Jan.	23.7 ± 0.13	-0-	0.23	- 0.23
5	+	Sept.	23.8 ± 0.12		0.51	
	0	Nov.	23.9 ± 0.55	+ 0.1	1.25	+ 0.74
	0	Jan.	24.2 ± 0.50	+ 0.3	0.62	- 0.63
6	+	Sept.	23.6 ± 0.06		0.23	
	+	Nov.	23.5 ± 0.11	- 0.1	0.31	+ 0.08
	+	Jan.	23.1 ± 0.06	- 0.4	0.27	- 0.04
7	+	Sept.	23.5 ± 0.02		0.07	
	+	Nov.	23.6 ± 0.14	+ 0.1	0.41	+ 0.34
	+	Jan.	23.5 ± 0.13	- 0.1	0.29	- 0.12
	+	Feb.	23.3 ± 0.06	- 0.2	0.13	- 0.16
8	0	Sept.	23.7 ± 0.04		0.15	
	+	Nov.	23.4 ± 0.16	- 0.3	0.46	+ 0.31
	+	Jan.	23.2 ± 0.02	- 0.2	0.07	- 0.39

* τ ± 95% Confidence Interval

** Standard deviation of plotted values about the trend line

TABLE XI

Effect of Surgical* Replacement of Radiotransmitters on the Subsequent Free-Running Circadian Rhythm of Body Temperature in Female Pocket Mice, Perognathus longimembris

Animal Number	Experiment Regimen	τ_{FR} Pre-Surgery (Hours)	τ_{FR} Post-Surgery (Hours)	$\Delta\tau$ (Hours)	$\Delta\phi$ (Hours)
2635	LL	26.40	25.50	-0.9	0
2856	DD**	23.16	23.75	+0.59	-1.7
3087	DD	23.58	23.67	+0.09	0
3135	DD	23.16	22.50	-0.66	-3.5
3050	DD	23.50	23.67	+0.17	+1.0
	DD	24.03	23.50	-0.53	0
2969	DD	23.92	23.58	-0.34	-1.0
2746	DD	23.00	23.75	+0.75	-1.0
3004	DD	24.16	24.25	+0.09	+1.0
3153	DD	24.25	23.58	-0.67	0
3168	DD	23.58	24.25	+0.67	0

*Mice anesthetized in DD; surgery under light; returned to DD within 20 minutes still anesthetized.

**Mouse transferred to LL prior to anesthesizing.

In terms of persistence and precision of a given τ_{FR}, mice studied over long periods tended to fall into three categories. First were mice whose τ_{FR} was relatively unchanged for long periods (Figure 22). Most cases fell in this category. Second were mice whose τ_{FR} tended to drift from less than 24 hours to greater than 24 hours over several week's time (Figure 23). Third were mice whose τ_{FR} was very imprecise and drifted in value (Figure 24). Mice in the third category frequently expressed multiple torpors in a single day leading to an impression of the rhythm breaking up into separate components each with a circadian property. Mice in the third category were rare.

The impression is left that while τ_{FR} is obviously persistent it is also very labile. The τ_{FR} expressed over a few days to weeks is characterized by varying degrees of imprecision reflected by the dispersion of ϕ_R's about the trend line. This could be interpreted as evidence for the presence of an underlying circadian pacemaker to which the ϕ_R's are imperfectly coupled.

2. Temperature Compensation. Five mice were maintained in constant dark at an ambient temperature (T_A) of 21°C followed by a step drop to 10°C. The τ_{FR} was derived in both regimes from times of arousal from torpor. The experiment was conducted during an earlier contract and formally published under this contract (2). The data are repeated here in the interest of summarizing key properties of the circadian rhythm of body temperature.

Representative data from one animal is presented in Figure 25 and a summary of data from 4 animals presented in Table XII. The relative independence of τ from metabolic rate is indicated by the ratio of τ at T_A = 21°C to τ at T_A = 10°C. The mean value of the ratio of period lengths was about 1.01. An animal which is torpid 3-4 hours a day at T_A = 21°C becomes torpid for 20 hours a day at T_A = 10°C. In Figure 24 the torpors are even more prolonged and may last as long as 3 days.

Figure 22. Persistence of the free-running rhythm of body temperature in Perognathus longimembris. Times of arousal from torpor are plotted on successive days. The time scale equals 48 hours and the data are double plotted. The mouse was maintained in constant dark and constant temperature ($20^{\circ} \pm 0.5^{\circ}C$) for 9 months. Phase shift experiments were undertaken during the tenth and eleventh month. Six hour heat pulses ($10^{\circ}C$) are indicated by a bar. Triangles indicate times of feeding and crosses times of telemeter transplant (see text).

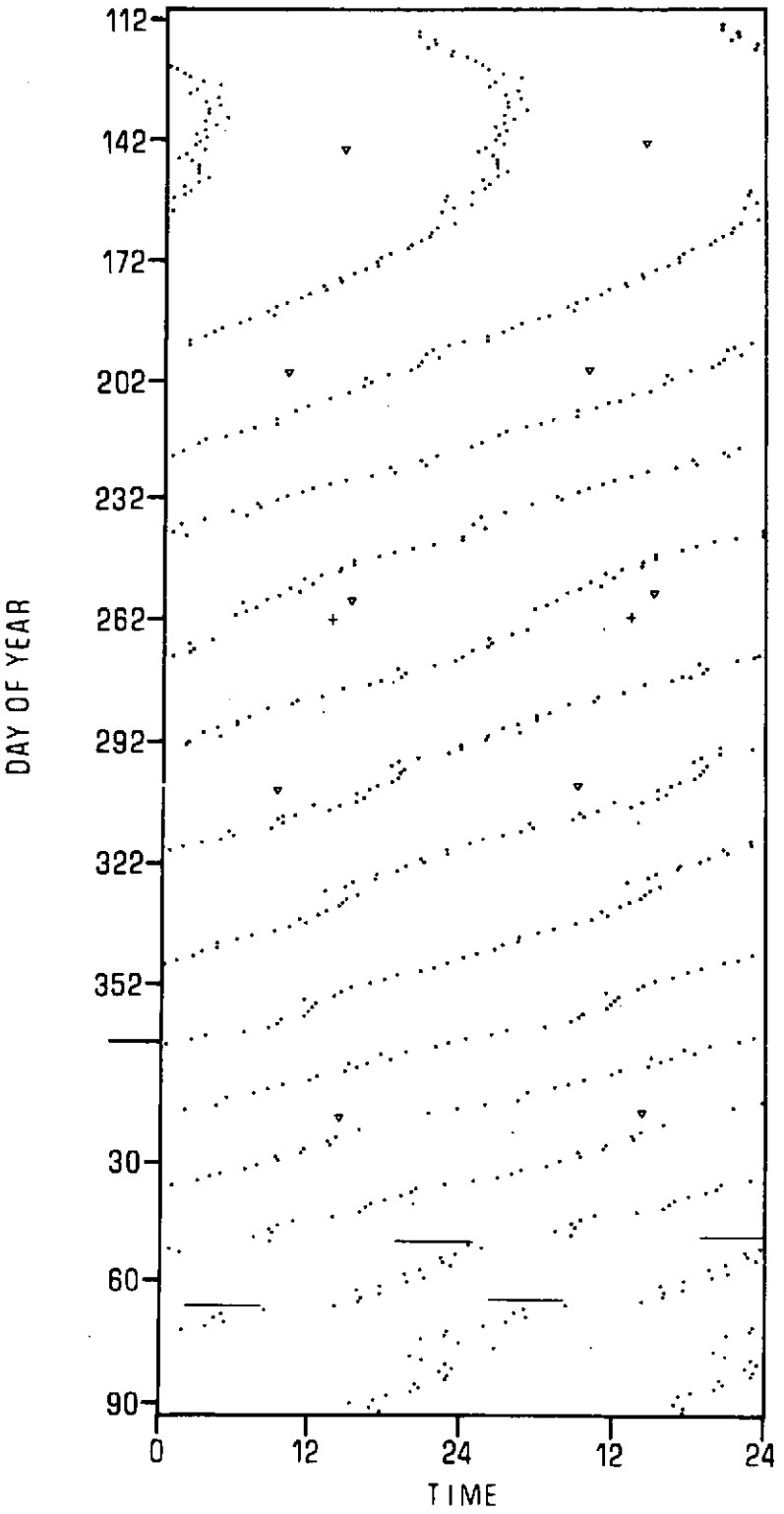
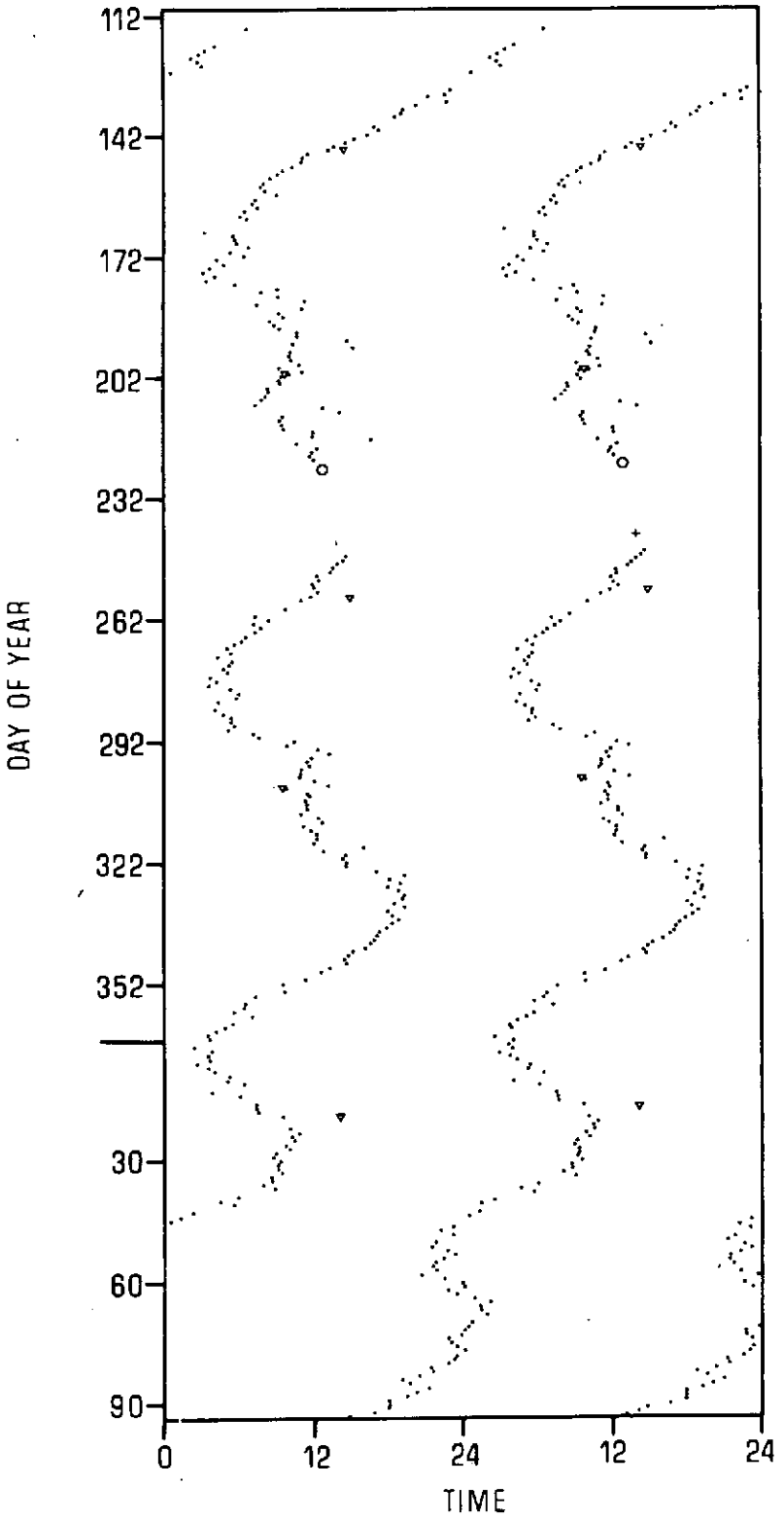

112
142
172
202
232
262
292
322
352
30
60
90
DAY OF YEAR
0
12
24
12
24
TIME

Figure 23. Persistence of the free-running rhythm of body temperature in Perognathus longimembris. Times of arousal from torpor are plotted on successive days. The time scale equals 48 hours and the data are double plotted. The mouse was maintained in constant dark and constant temperature ($20^{\circ} \pm 0.5^{\circ}C$) for 9 months. Triangles indicate times of feeding, open circles denote telemeter failure and crosses times of telemeter transplant (see text).


112
142
172
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232
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322
352
30
60
90
DAY OF YEAR
0
12
24
12
24
TIME

Figure 24. Persistence of the free-running rhythm of body temperature in Perognathus longimembris. Times of arousal from torpor are plotted on successive days. The time scale equals 48 hours and the data are double plotted. The mouse was maintained in constant dark and constant temperature ($20^{o} \pm 0.5^{o}C$) for 9 months. Triangles indicate times of feeding, open circles denote telemeter failure and crosses times of telemeter transplant (see text).

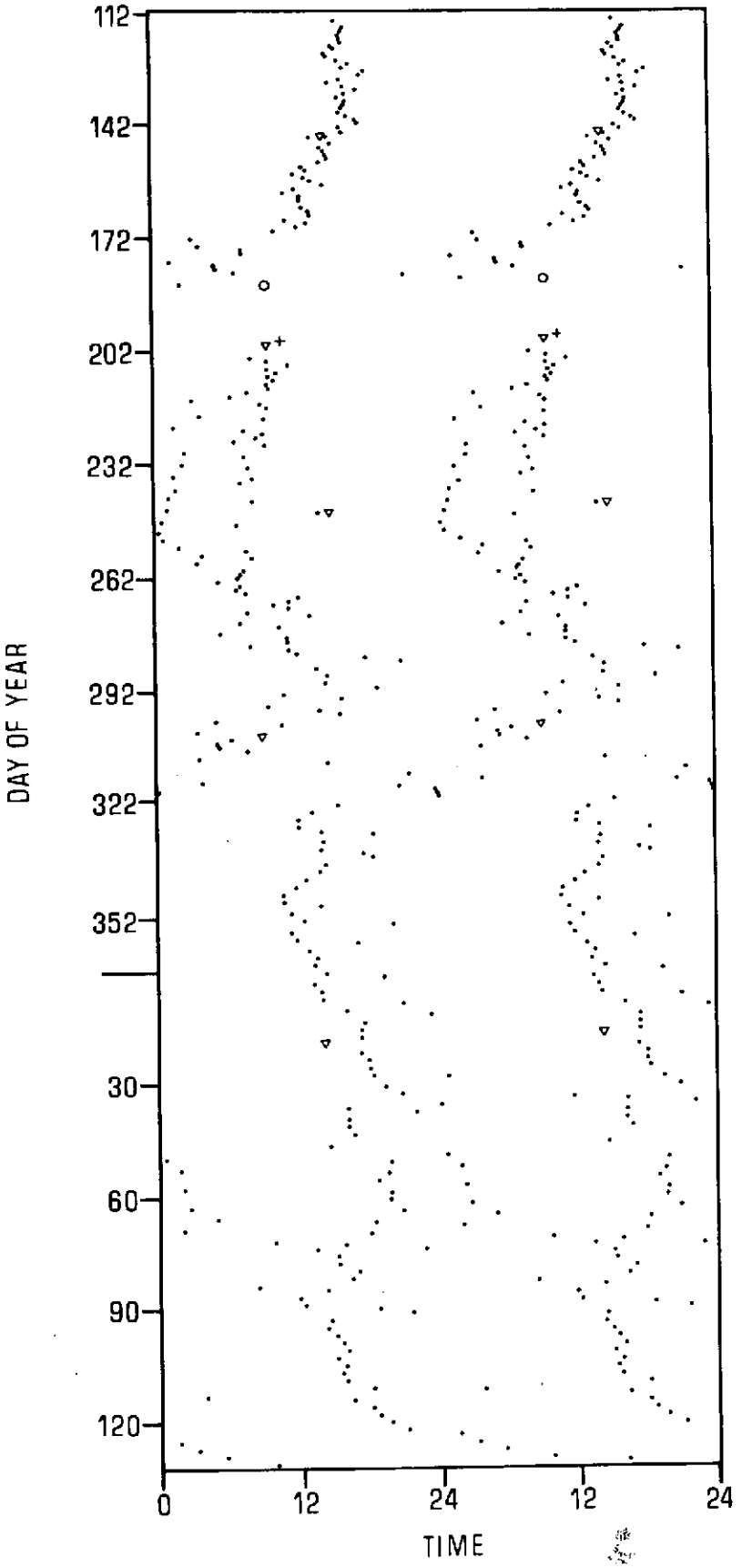
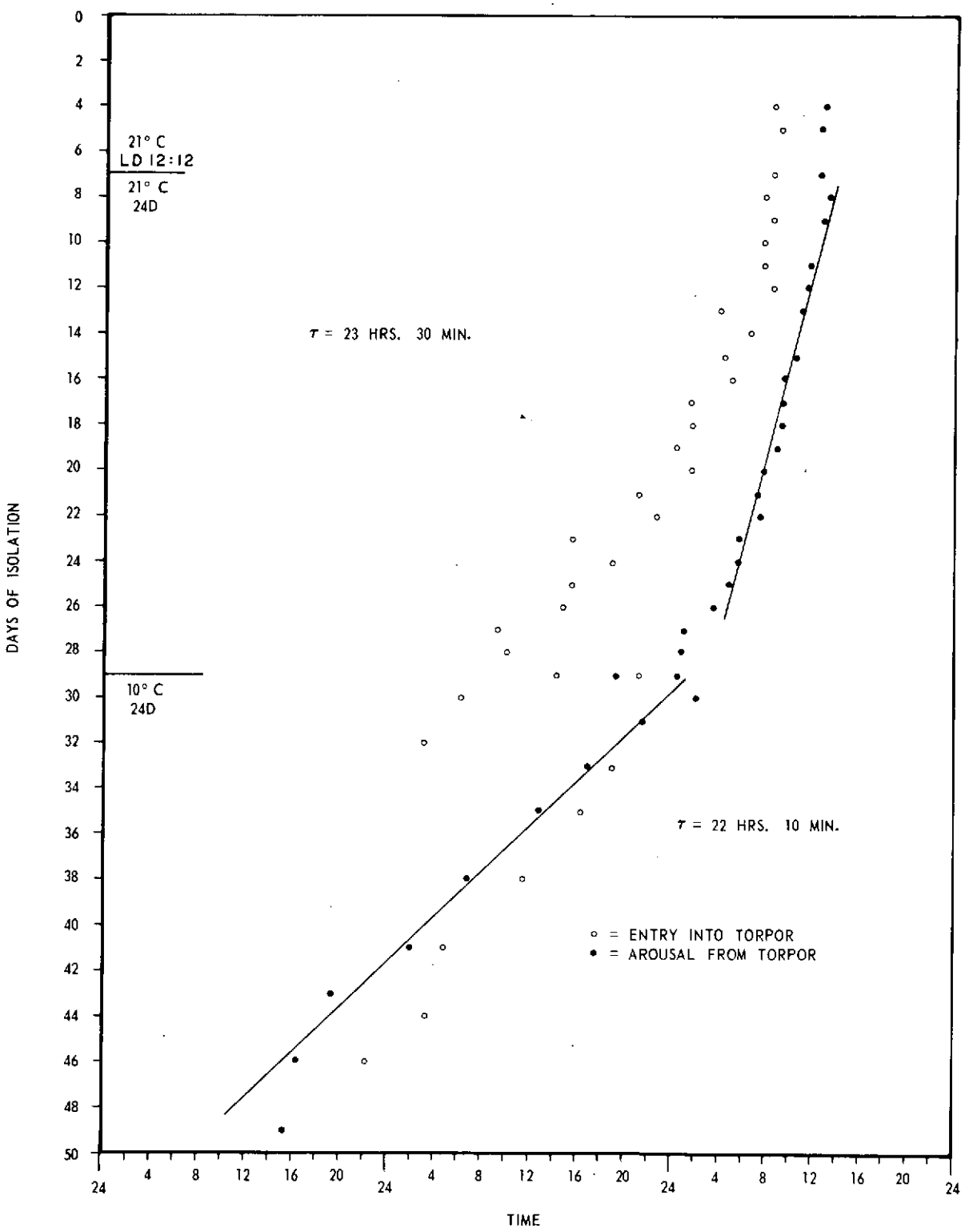

112
142
172
202
232
262
292
322
352
30
60
90
120
DAY OF YEAR
0
12
24
12
24
TIME

Figure 25. Demonstration of temperature compensation of the free-running circadian rhythm of body temperature in Perognathus longimembris. Times of entry (open circles) and arousal (dots) from torpor are plotted on successive days in two different temperature regimens (20°C and 10°C). Torpor expressed in low temperature regimens is characteristically long (> 24 hours). Days for which no data are given are days in which arousal from torpor did not occur. Long torpors also present an illusion in the plotted data of arousal preceeding entry.


21° C
LD 12:12
21° C
24D
τ = 23 HRS. 30 MIN.
10° C
24D
τ = 22 HRS. 10 MIN.
○ = ENTRY INTO TORPOR
● = AROUSAL FROM TORPOR
DAYS OF ISOLATION
TIME
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
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50
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4
8
12
16
20
24
4
8
12
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20
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12
16
20
24

TABLE XII

Effect of Ambient Temperature on the Free-Running Circadian Period of Body Temperature in Perognathus longimembris Maintained in Constant Dark

Animal	τ (hr) $T_A = 21^\circ$	τ (hr) $T_A = 10^\circ$	Transients (days)	$\tau_{21^\circ}/\tau_{10^\circ}$
1	23.5	22.2	1	1.06
2	23.1	22.2	1	1.04
3	23.7	23.5	8	1.01
4	23.1	24.4	4	0.95

Note: τ was determined from time of arousal from torpor.

In other words, while the animal is torpid its body temperature equals or closely approximates T_A, but τ is relatively unaffected.

All animals were affected by the change in T_A. Two stabilized at a new τ within 1 day. One showed multiple drops (eight) in body temperature on the day following the temperature change, but stabilized at a new τ after 4 days. One animal showed transients for 8 days before stabilizing at essentially the same τ expressed at $T_A = 21^{\circ}$.

3. Effect of Deuterium Oxide on τ_{FR}. Deuterium oxide added to the drinking water (3) or culture media (4) of a variety of organisms produces a lengthening of τ_{FR} proportional to the concentration of D_2O, and comparable to τ_{FR} lengthening produced by low temperature. Perognathus longimembris is unusual in that temperature compensation of τ_{FR} is manifest by shortening rather than lengthening of τ_{FR} (5). If the premise stated above is true, then D_2O administered to pocket mice should shorten the τ_{FR}.

Perognathus longimembris does not drink water. However, various concentrations of D_2O injected I. P. showed no evidence of toxicity. Ten animals with implanted temperature telemeters were selected for study. Since this was intended as a pilot experiment to verify procedures, animals implanted with telemeters nearing the end of their battery life were used. In the course of the experiment, four telemeters failed.

Each animal was injected I. P. with 1 ml of normal saline. All manipulations were done under low intensity light from a ruby red photographic safelight. Two weeks later, after determining that the saline injection had not perturbed the τ_{FR}, each animal was again injected with 1 ml of normal saline in which H_2O was replaced with D_2O. The free fluid volume in P. longimembris is estimated between 2-3 ml. Assuming that the D_2O was rapidly absorbed, the concentration of D_2O in the blood stream was estimated to be between 20-30% of the plasma volume.

It was desirable to confirm that the animals used in this experiment indeed did evidence a shortening of τ_{FR} at reduced ambient temperature. The expected life of the telemeters, however, was very short. Therefore, as soon as the data suggested that the effects of the D_2O injection were over, and that the animals were in, or approaching, a new steady state, the ambient temperature was dropped from 20°C to 10°C. Twenty-one days later ambient temperature was raised back to 20°C and maintained until completion of the experiment.

The animals were housed in a constant temperature room which was entered on four occasions: twice for the experimental treatment and twice for feeding. Coincident with the last feeding, the cooling unit on the roof of the constant temperature room was repaired. This activity lasted for two hours and could have disturbed the animals. During repairs the ambient temperature rose from 10°C to 15°C where it remained for about one hour before dropping back to 10°C.

Plots of data obtained from one animal are presented in Figures 26 and 27. In Figure 26 only time of arousal from torpor (ϕ_R) on successive days is plotted. In Figure 27 total time in torpor is indicated by a solid bar on successive days. Time of injection of normal saline is indicated by "W"; time of injection with D_2O by "D"; time of feeding by "F"; change in temperature regime by "T"; and time of cooling system repair by "R." Days for which no data are given represent days in which torpor was not expressed.

The animal depicted was the only animal which may have responded to the normal saline injection (W) by shortening the τ_{FR}. The nine other animals appeared unaffected.

It should be noted that the D_2O injections were given at different circadian times. It is not evident, however, that that variable influenced the data.

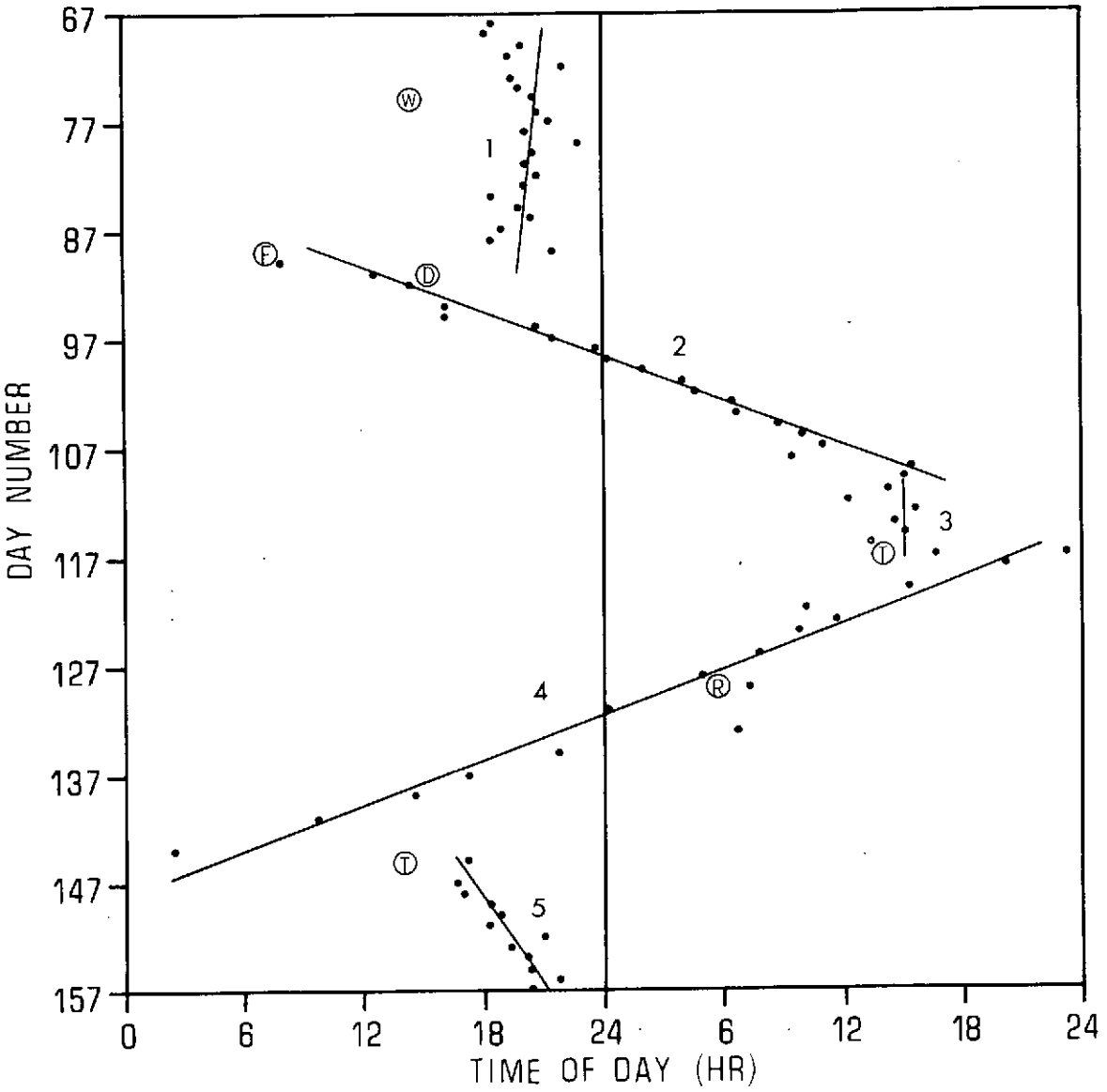


Figure 26. Effect of injections of deuterium oxide compared to effects of temperature compensation of the free-running circadian rhythm of body temperature in Perognathus longimembris. Only times of arousal from torpor (dots) are plotted on successive days to clarify the sequence of events. The same data showing duration of torpor are presented in Figure 27. W indicates a control I. P. injection of 1 cc of normal saline. D indicates an I. P. injection of 1 cc of normal saline with H_2O replaced by D_2O. F indicates addition of food. T indicates beginning and end of a temperature regimen of $10^{\circ} \pm 0.1^{\circ}C$. Cage air temperatures were maintained at $20^{\circ} \pm 0.1^{\circ}C$ at all other times. An accidental two hour increase of $5^{\circ}C$ occurred during the $10^{\circ}C$ regimen (R). Mouse was fed during equipment repair.

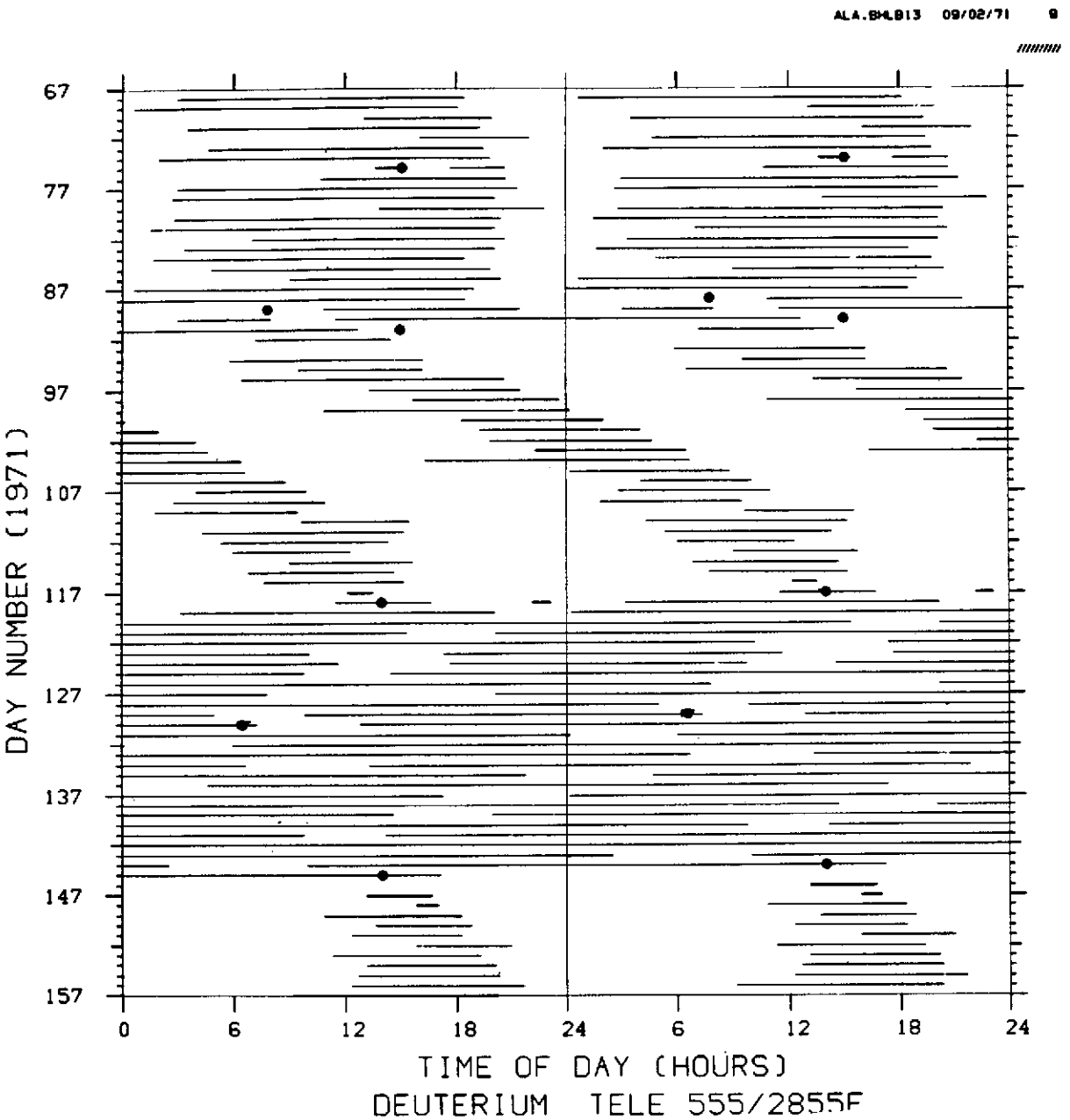


Figure 27. Effect of injections of deuterium oxide compared to effects of temperature compensation of the free-running circadian rhythm of body temperature in Perognathus longimembris. These are the same data presented in Figure 26 but duration of torpor on successive days is indicated by a bar. Filled circles indicate time of events described in Figure 26.

Four of the ten animals expressed τ_{FR} of greater than 24 hours at the beginning of the study, and seven showed τ_{FR} less than 24 hours. Lengthening of τ_{FR} in the presence of D_2O occurred in all 10 animals but was most pronounced in those with preinjection τ_{FR} of less than 24 hours.

In view of reports in the literature that response to D_2O is proportional to D_2O concentration, it is interesting that for this method of administration the response seems to be all or none. There was only slight evidence of the period gradually changing as the D_2O concentration changed with time. This phenomenon may be related to the pocket mouse's peculiar water metabolism, but it would be interesting to try the protocol on Peromyscus.

If the duration of the D_2O effect can be related to water metabolism, then the data suggests that the biological half life of 1 ml of water in the pocket mouse is 5 or 6 days.

The results of this pilot experiment indicate that D_2O is probably not effecting the temperature compensation mechanism of P. longimembris since it produces a lengthening rather than a shortening of τ_{FR}. The τ_{FR} following administration of D_2O resembles τ_{FR} expressed by pocket mice in constant light.

4. Effect of Length of Torpor on τ_{FR}. Spontaneous torpors ranging in length from 1 hour to 86 hours have been observed. Duration of torpor and pattern of successive torpors are highly variable (Table XIII). There is little evidence that either expression of torpor or duration of torpor affect τ_{FR} (Tables IX, X, XIV). There is a slight tendency for τ_{FR} to shorten with increasing time in torpor (Figure 28). That latter observation is consistent with observation made during studies of temperature compensation in which long torpors were observed during times of low ambient temperature.

C. Discussion

Characteristics of the free-running circadian period of P. longimembris are similar to those of other nocturnal mammals reported in the literature (6,

TABLE XIII

Mean Time/Day in Torpor (Hours) Exhibited by Twelve Pocket Mice Selected for Experiment S-071

Animal	HU/LMU Initial Selection $\bar{X} \pm$ S. D.	N*	HU/LMU Pre-Launch $\bar{X} \pm$ S. D.	N	Space Experiment Hardware Flight (CPE 2) $\bar{X} \pm$ S. D.	N
3301 M	7.78 ± 3.11	19	2.81 ± 0.79	19	7.19 ± 0.85	6
3081 M	11.59 ± 3.23	32	9.93 ± 1.66	21	7.74 ± 4.21	6
3337 M	13.29 ± 7.22	21	5.42 ± 1.64	19	8.63 ± 3.37	5
3295 F	10.06 ± 2.34	19	4.48 ± 1.62	21	8.57 ± 1.31	5
3341 M	8.79 ± 2.10	21	5.62 ± 0.80	22	6.42 ± 2.18	6
3307 M	11.07 ± 2.63	20	5.81 ± 1.17	20	7.40 ± 0.28	5
					Space Experiment Hardware Control (CPE 1)	
2984 M	9.65 ± 3.89	29	5.34 ± 1.28	21	9.39 ± 3.10	30
3291 M	6.14 ± 1.12	16	4.30 ± 0.58	22	5.55 ± 0.64	27
3061 M	8.14 ± 2.76	14	6.42 ± 1.90	21	8.60 ± 2.15	28
2909 M	5.20 ± 0.77	30	6.06 ± 1.05	19	7.40 ± 2.72	22
3375 M	10.84 ± 3.40	20	7.73 ± 1.28	21	14.50 ± 2.11	29
3264 F	5.09 ± 2.28	18	6.79 ± 1.06	21	10.06 ± 2.23	27

*N = Number of days torpor was expressed. Duration of experiments were 30 days for initial selection; 21 days pre-launch; 6 days CPE 2; and 30 days CPE 1.

TABLE XIV

Relationship of Duration of Torpor to Length and Precision of the Free-Running Period of Body Temperature in 24 Pocket Mice Selected for Experiment S-071

Animal Number and Sex	Ave. Torpor/Day (Hrs & Min)	τ_{FR} ± 90% C.I. (Hours)	Dispersion (± Hours)	
3320 M	0148	24.1±0.1	0.6	
2926 M	0152	23.4±0.4	1.1	
3301 M	0226	24.4±0.1	0.8	
3405 M	0236	23.8±0.1	0.8	
3196 M	0315	23.4±0.1	1.3	
2960 F	0342	23.7±0.1	1.0	
	Average 0309	23.8	0.8	(Range 0.6-1.3)
2979 M*	0351	27.7±0.5	4.5	
3351 M	0404	23.7±0.1	0.6	
3291 M	0419	23.5±0.1	0.9	
3084 F	0423	23.6±0.1	1.0	
3295 F	0444	23.5±0.1	1.5	
3352 M	0453	24.0±0.1	1.5	
3369 M	0502	23.6±0.1	1.2	
	Average 0434	23.7	1.1	(Range 0.6-1.5)
2984 M	0521	23.4±0.0	0.4	
3337 M	0524	23.5±0.1	0.6	
3307 M	0528	23.8±0.1	0.7	
3341 M	0540	24.3±0.1	0.7	
2909 M	0556	23.7±0.1	1.0	
3061 M	0632	23.2±0.0	0.4	
	Average 0544	23.7	0.6	(Range 0.4-1.0)
3264 M	0655	23.4±0.1	0.8	
3375 M	0742	23.8±0.0	0.6	
3081 M	0946	25.5±0.1	0.8	
3339F	1057	23.9±0.1	0.8	
3261 F	1457	23.6±0.1	1.6	
	Average 0956	23.6	0.9	(Range 0.8-1.6)

*Not included in averages

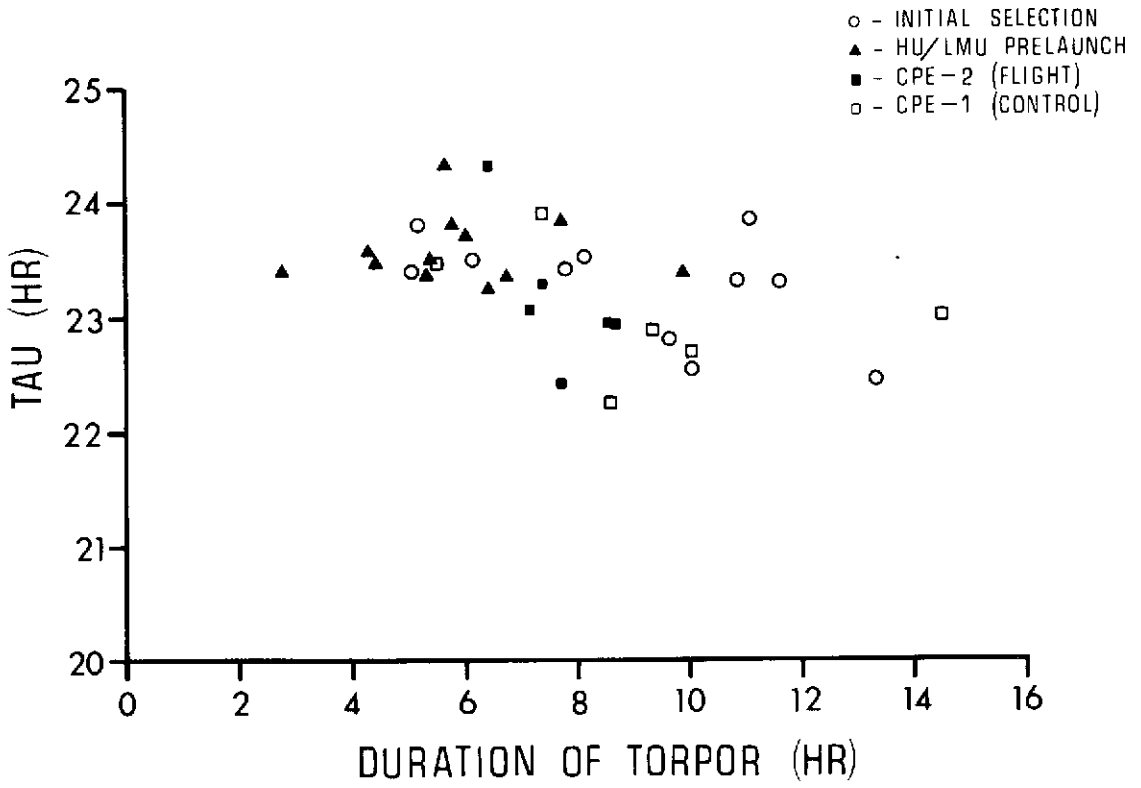


Figure 28. Relationship of the free-running circadian period of body temperature (τ) to the duration of torpor in Perognathus longimembris. Data were collected at three different times from animals selected for Experiment S-071. Experiment conditions were essentially identical and maintained constant dark and constant temperature ($20^{\circ} \pm 0.1^{\circ}C$). "Initial Selection" and "HU/LMU Prelaunch" were done with the same equipment.

The "free-running" circadian period (τ_{FR}) by definition, is the period expressed by an organism held in a constant environment shielded from entraining agents (zeitgebers). It is not a fixed characteristic of individual animals but is limited to a narrow range of values (nominally 23-25 hours). The precise τ_{FR} expressed at any time is a function of the environment immediately preceding the steady state free-run and the physiological state of the animal. Transients almost always precede attainment of a new steady state. The phase of a free-running period may be shifted by a single exposure to an entraining stimulus and the subsequent τ_{FR} is characteristically different in length but does not necessarily degrade in precision. These general traits have been verified for Perognathus longimembris while housed both in laboratory monitoring equipment and space experiment hardware.

The persistence of a given τ_{FR} was shown to be good over periods of as long as a year for most animals. Some mice, however, slowly drifted from short τ_{FR} to long τ_{FR} and back again over several week's time. The pattern was reoccurring. The overall impression was that the τ_{FR}, while persistent, is very labile in this species.

Perognathus longimembris is unusual, but not unique, in that τ_{FR} shortens with lowered ambient temperature. A similar response has been reported for the Dormouse (Glig glis) (8). Both species express torpor and/or hibernate.

An hypothesis existing in the literature suggested that deuterium oxide acted on the temperature compensation mechanism of organisms to slow the circadian system. A pilot experiment was undertaken with P. longimembris which did not support the hypothesis. That same experiment yielded observations suggesting that the biological half life of water in P. longimembris is 5-6 days.

III. ENTRAINMENT OF THE CIRCADIAN RHYTHM OF BODY TEMPERATURE TO LIGHT CYCLES

A. Methods

Experiment conditions were the same as described in II-A with one exception. Boxes containing the monitoring cages could be illuminated by a 25 watt tubular incadescent bulb. The light bulb was encased in a glass water jacket through which water was circulated at the same temperature as the constant temperature room (20°C). Cage to light distance could be adjusted to provide from 140 to 1160 lux intensity evenly throughout the cage. Lights were turned on and off by an external timer to produce desired photoperiods.

When the lights were on, in spite of the water jacket and air flowing through the cage boxes, temperatures at the surface of the bedding increased by as much as 2.1°C. Measurements were taken with unshaded thermisters. Shaded thermisters in the same area showed no increase in air temperature. There was no evidence, under these circumstances, of the temperature rises associated with lights-on affecting the response of animals to light.

B. Results

1. Entrainment to 24 hour light cycles. Perognathus longimembris clearly entrained to light (Figure 29). The phase angle (ψ) of the circadian rhythm of body temperature, as estimated by times of arousal from torpor, varied with light to dark ratio (L:D) in a manner similar to other organisms (Figure 30). Entrainment to light cycles was accomplished at light intensities between 230 and 1160 lux. Attempts to entrain mice to lower and higher intensities were not made. It is not evident from the data that phase angle was dependent on light intensities between 230 and 1160 lux (Figure 30).

2. Entrainment to Other Than 24 Hour Light Cycles. Only one of five animals successfully entrained to a 22 hour day (L:D 9:13). Relative coordination was demonstrated by the other four (Figure 29).

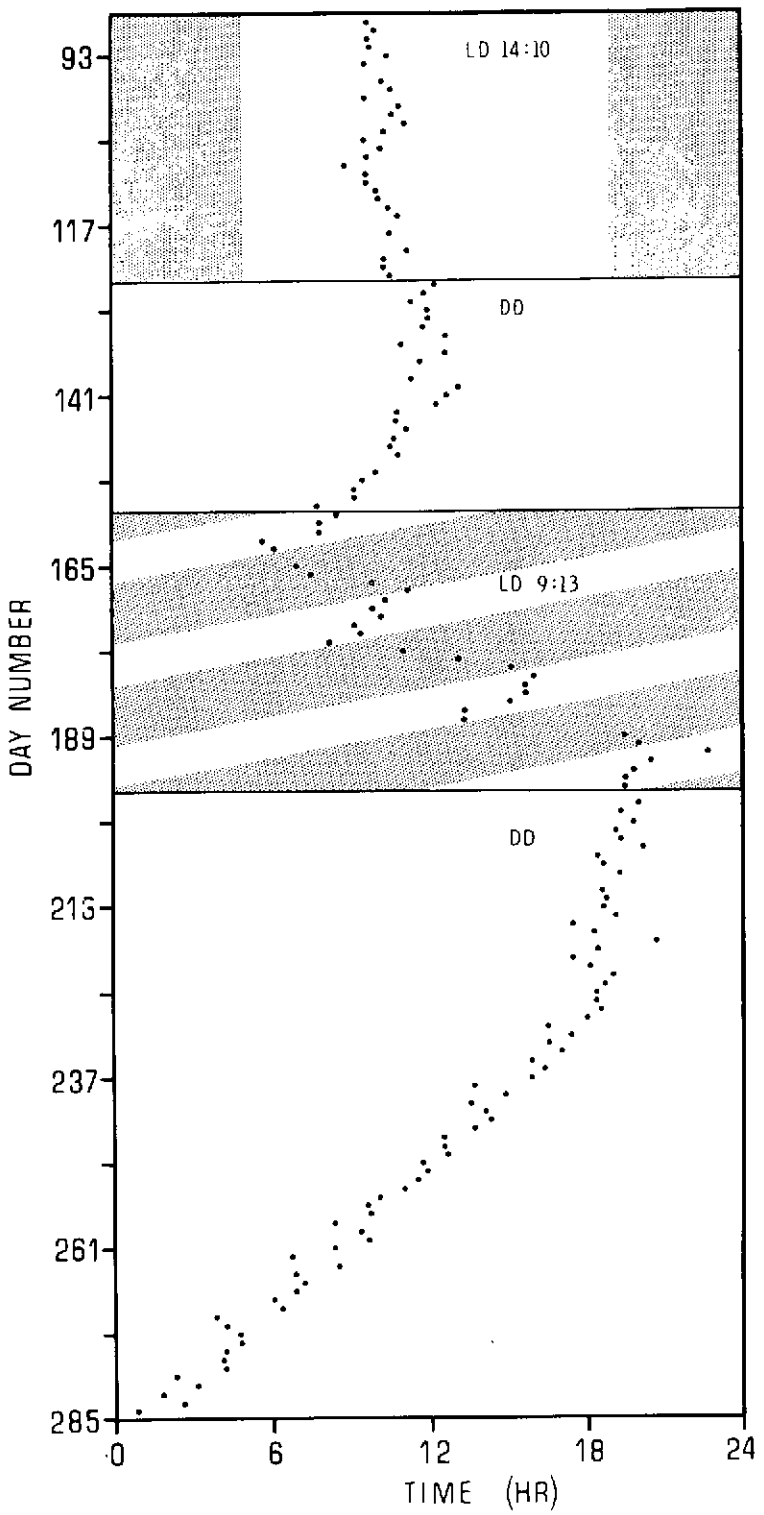
One mouse was exposed to a 90 minute entraining period (L:D 15 minutes: 75 minutes at 1160 lux). The experiment was undertaken to provide insight into the possible affects of undefined zeitgebers associated with the 90 minute orbital period of Skylab (Table XV). The mouse entrained to a single 15 minute light pulse every 24 hours, but appeared to interpret the 15 minutes of light every 90 minutes as constant light (LL). Under LL conditions, however, the circadian period became even longer. Data from this single experiment are difficult to interpret. The inference is that a zeitgeber applied once every 90 minutes may be interpreted as a constant rather than interrupted stimulus by P. longimembris.

3. Attempts to Improve the Precision of τ_{FR}. The precision of τ_{FR} was measured by the dispersion of phase reference points (times of arousal from torpor). Dispersions of between ± 0.5 and ± 1.0 hours were characteristic of the data. Dispersions of greater than ± 1.0 hour, however, were not unusual. A question was posed as to whether the dispersion observed in a given animal was a "fixed" characteristic of its circadian system. If not, could dispersion values be used to define steady states.

One experiment was undertaken with particular attention to changing the dispersion values for a given animal. Eleven mice were maintained in DD for 21 days, followed by 21 days in LL, followed by 21 days in DD (Table XVI). Following the transition from DD to LL dispersion became less in 5 mice and greater in 6 with no change in the mean. Following the transition from LL to DD the dispersion became less in 5 of 8 animals with a change in the mean from ± 1.10 hours to ± 0.89 hours.

The data suggest that dispersion is not a "fixed" characteristic. Possibly a resonance experiment would reduce the dispersion to a minimum. Animals chosen for Experiment S-071 were selected partially on the basis of having dispersions of less than ± 1.0 hours prior to insertion in the space experiment hardware. The mean dispersion of animals selected for space flight was ± 0.45 hours with a range of ± 0.21 to ± 0.84 hours (Table II, CPE 2).

Figure 29. Entrainment of the circadian rhythm of body temperature to light cycles in Perognathus longimembris. Times of arousal from torpor (dots) are plotted on successive days in lighting regimens of LD 14:10, 9:13 and DD. Duration of light (230 lux) is indicated by shading. Note entrainment to LD 14:10; relative coordination to LD 9:13; free runs in DD; and differences in duration of transients following release to DD. Cage air temperature was controlled at $20^{\circ} \pm 0.1^{\circ}C$.


LD 14:10
DD
LD 9:13
DD
93
117
141
165
189
213
237
261
285
DAY NUMBER
0
6
12
18
24
TIME (HR)

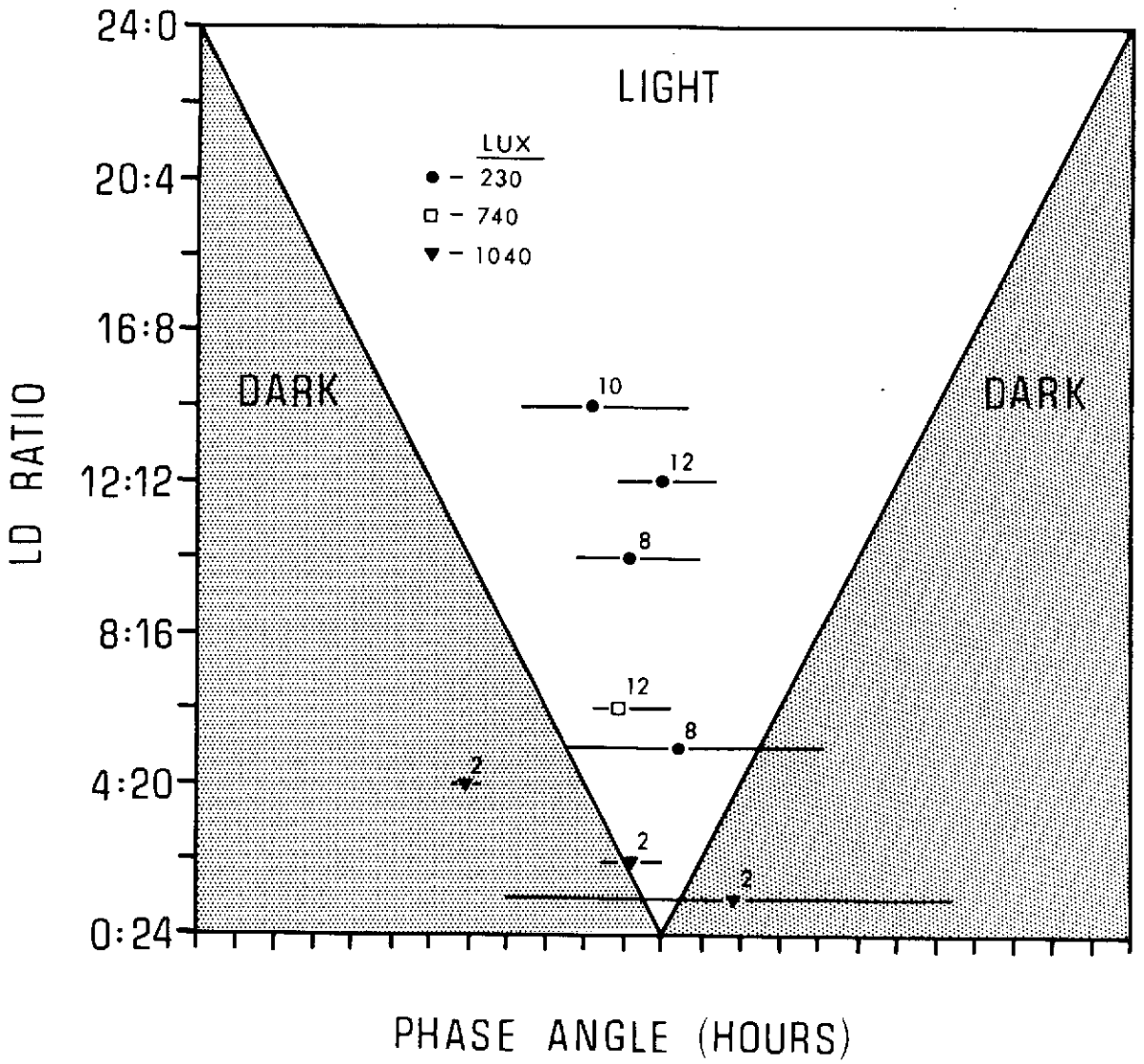


Figure 30. Changes in the phase angle of the circadian rhythm of body temperature in response to entrainment to different lighting regimens. Time of arousal from torpor was used as the phase reference. Symbol denotes average value for phase reference point; bar denotes range of values; and numeral above average gives number of animals in the sample. All data were collected with cage air temperature controlled to $20^{\circ} \pm 0.1^{\circ}C$.

TABLE XV

Effect of Exotic Photoperiods on the Circadian Period of Body Temperature in One Female *Perognathus longimembris*

Experiment* Conditions	τ ± 95% C.I. (Hours)	Dispersion (Hours)**
L:D 0.25:23.75 hrs	24.00 ± 0.03	0.56
L:D 15:75 min	26.52 ± 0.08	0.55
LL	27.56 ± 0.30	1.84

*Mouse environment maintained at $20^{\circ} \pm 0.1^{\circ}C$.

**Dispersion equals standard deviation of phase reference points about the trend line.

TABLE XVI

Effect of Constant Light and Constant Dark on the Expression of the Circadian Period of Body Temperature in Perognathus longimembris

Animal Number	Constant Dark τ_{FR} ± 95% C.I. (Hours)	Constant Dark Dispersion* (± Hours)	Constant Light τ_{FR} ± 95% C.I. (Hours)	Constant Light Dispersion (± Hours)	Constant Dark τ_{FR} ± 95% C.I. (Hours)	Constant Dark Dispersion (± Hours)
1	23.75 ± 0.08	0.96	25.86 ± 0.10	1.40		
2	23.86 ± 0.04	0.79	24.95 ± 0.20	2.15		
3	24.15 ± 0.11	1.13	27.08 ± 0.18	0.64	24.39 ± 0.26	0.61
4	23.78 ± 0.13	1.06	28.00 ± 0.20	0.59	24.25 ± 0.18	0.98
5	23.84 ± 0.11	1.31	26.54 ± 0.24	0.49	24.20 ± 0.18	1.16
6	24.38 ± 0.08	1.01	27.37 ± 0.41	1.36	24.72 ± 0.14	0.70
7	24.25 ± 0.08	1.07	26.79 ± 0.42	1.75	24.42 ± 0.31	1.46
8	23.91 ± 0.09	1.15	27.67 ± 0.24	0.66	24.22 ± 0.29	1.42
9	23.83 ± 0.11	1.29	29.11 ± 0.19	0.55	24.89 ± 0.19	0.78
10	24.51 ± 0.62	0.90	26.08 ± 0.13	0.99	23.50 ± 0.16	0.57
11	23.67 ± 0.11	0.92	27.24 ± 0.50	1.53	24.48 ± 0.06	0.31
Mean	23.99 ± 0.14	1.05	26.97 ± 0.26	1.10	24.34 ± 0.20	0.89

*Dispersion is the standard deviation of phase reference points about the trend line.

C. Discussion

The characteristics of entrainment of the circadian rhythm of body temperature to light cycles appear the same as reported for other nocturnal species. Efforts to improve the precision of τ_{FR} were not effective but did indicate that dispersion values were not fixed characteristics of the τ_{FR}. τ_{FR} in DD following an LL experience was longer than τ_{FR} in DD before the LL experience. The latter observation is further indication of the fact that τ_{FR} at any given time may be influenced by prior treatment.

Efforts to entrain P. longimembris to short day lengths lead to the conclusion that the shortest τ that can be expressed is approximately 22 hours. τ in LL is characteristically long. The maximum observed was 29.11 hours. Based on these observations it can be predicted that a phase response curve to light will show a maximum phase advance of about 2 hours and a maximum phase delay of about 5 hours.

IV. ENTRAINMENT OF CIRCADIAN RHYTHM OF BODY TEMPERATURE TO TEMPERATURE CYCLES IN THE ENVIRONMENT

A. Methods

Experiment conditions were the same as described in II-A with one exception. A nichrome heating element in the center of a transite tube was mounted on the air inlet of each cage box. Current to the heater was supplied through a proportional controller which was turned on and off by an electric timer. A thermister probe was located on the animal cage in a position representative of the air temperature which the animal sensed, and served as the sensor for the controller. Additional thermisters were used to monitor changes in air temperature during experiments. The overall system provided a rapid rise in temperature (10^{o}C in 20 minutes) and held at any given setting within $\pm 0.1^{o}$C. At the end of the stimulus the temperature dropped to within 10% of the base line in approximately 40 minutes.

Temperature rise time could be varied by controlling power to the heating element through a variable transformer.

Observations of entrainment to quite low amplitude temperature cycles caused concern that something associated with the experiment configuration other than temperature might be causing entrainment. As a consequence, the experiment was repeated in a different configuration. A water jacketed cage was constructed with the ventilating air supplied to the cage through a copper tube heat exchanger placed in the water jacket. Except for the top, the entire cage assembly was surrounded by a four inch thick bat of fiber glass and was placed in a small laboratory incubator. Water was circulated through the water jacket from an external water bath whose temperature was controlled by switching to a "hot" or "cold" thermostat. Cage air temperature was monitored by a thermistor placed in the air stream near the inlet below the cage floor. This configuration also provided a rapid rise in temperature (~20 min) and held at any given setting within ± 0.1°C. Body temperature data were collected as before (See II-A.). Data obtained in the original experiment configuration were verified and both systems were used to obtain the data reported herein.

Animals in constant dark were exposed to daily rapid temperature rises of six hours duration. Amplitude of the temperature rises ranged from 1.5°C to 10°C depending upon the experiment. Except for programmed temperature excursions, cage air temperature was held at 20° ± 0.1°C.

B. Results

1. Entrainment to Temperature Cycles. *Perognathus longimembris* entrained to daily 6 hour exposure to rapid rises in ambient temperature. Thirteen of thirteen animals showed entrainment to thermoperiodic regimens of 3°, 5°, and 10°C (Figure 31). Two of three animals entrained to 1.5°C rises in temperature. One animal subjected to 1.1°C rises did not entrain, which suggests that 1.5°C is close to the threshold. It can be argued that since *P. longimembris* is heterothermic (i.e. express torpor) that it is to be

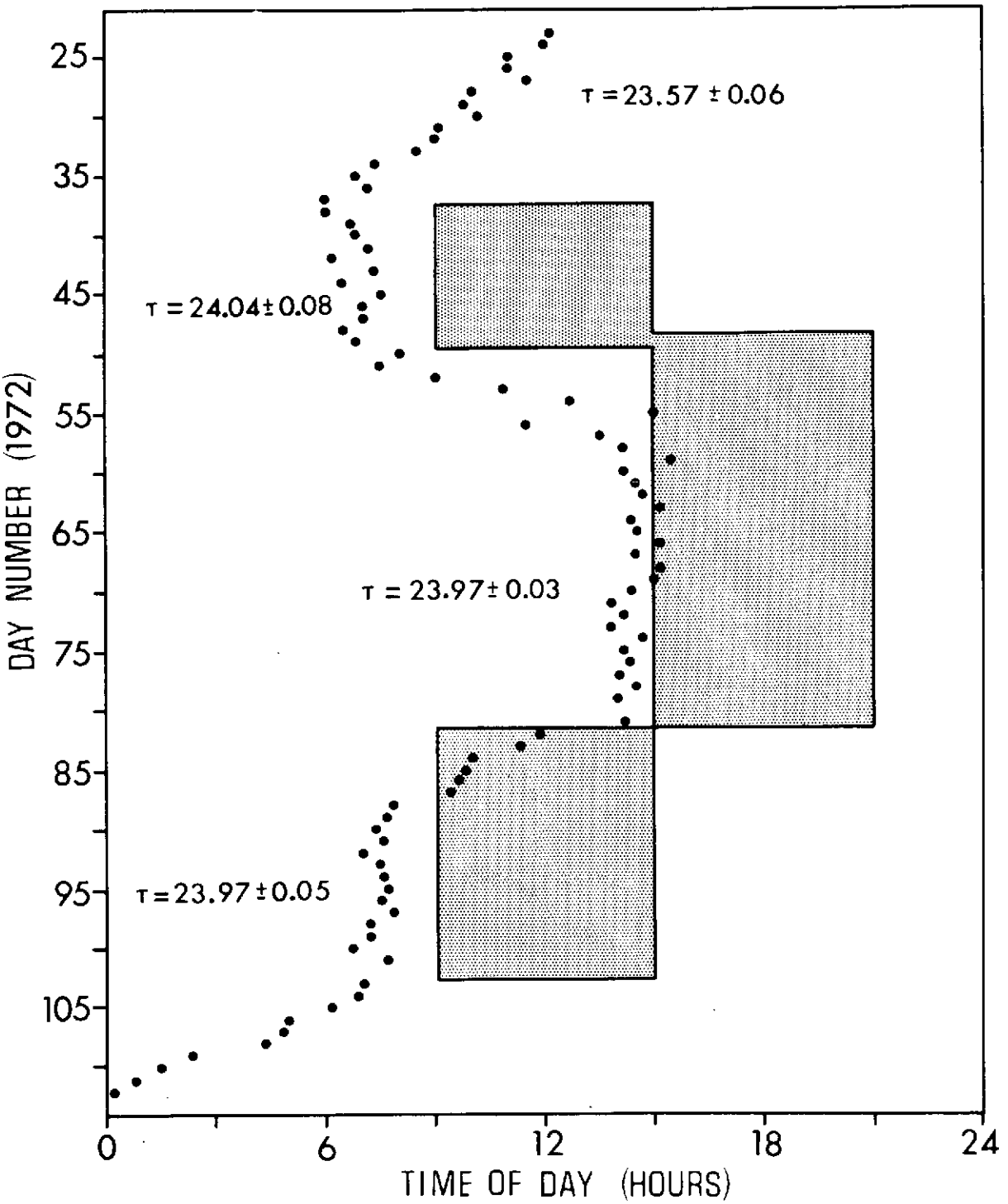


Figure 31. Entrainment of arousal from torpor in Perognathus longimembris by cyclic changes in ambient temperature. Dots indicate time of arousal from torpor on successive days. Circadian period (τ) is given ± 95% confidence internal. Shaded box demarks increase in air temperature from 20° ± 0.1°C to 23° ± 0.1°C. Experiment conducted in constant dark.

expected that it would entrain to temperature. However, while temperature pulses administered during torpor often will initiate arousal, it is clear from Figure 31 that entrainment for that animal involved anticipation of the temperature rise. Further the phase response curve presented (Figure 35) shows that temperature pulses are effective after arousal from torpor when the animal is showing thermoregulation more "typical" of a homeotherm. This implies that the temperature stimulus does not effect the circadian pacemaker directly but rather that the temperature is sensed and transduced to neural information.

2. Relationship of Intensity to Duration of Temperature Stimulus. Demonstration of entrainment of body temperature rhythm to cyclic changes in ambient temperature led to the question of whether entrainment to temperature exhibited the same properties as entrainment to light. The duration of temperature stimulus is plotted against the amplitude (intensity) of temperature stimulus in Figure 32. The shape of the curve is similar to curves representing the relationship of duration of light stimulus to intensity.

The implications of the data are that a fairly discrete amount of energy must be deposited to elicit a response. The data in Figure 32, while preliminary, indicated that a temperature rise of $10^{o}C$ elicits a response whether applied for 1 hour or 6 hours. A $10^{o}C$ rise in temperature, therefore, was accepted as representing "saturation" and used in defining a phase response curve to temperature (Figure 35).

3. Relationship of Circadian Period of Body Temperature and Its Phase Angle to the On-Set of the Temperature Cycle. The phase angle (ψ) of the circadian rhythm of body temperature was plotted as a function of τ_{FR} expressed by each animal after release from the entraining temperature cycle (Figure 33). While variable, the data show a trend for a more positive

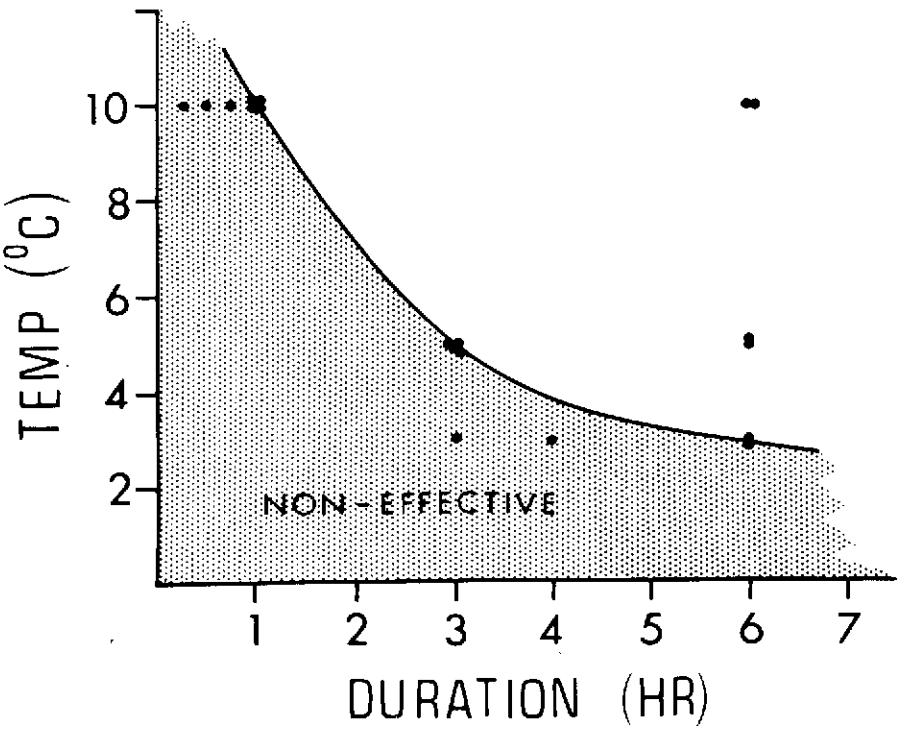


Figure 32. Duration and amplitude of an increase in ambient temperature required to produce a change in phase of the circadian rhythm of body temperature in Perognathus longimembris. Except for programmed temperature rises experiments were conducted in constant dark and constant temperature ($20^{\circ} \pm 0.1^{\circ}C$). All temperature rises were administered at CT 16. Phase changes were derived from steady state free-running periods determined before and after stimulus.

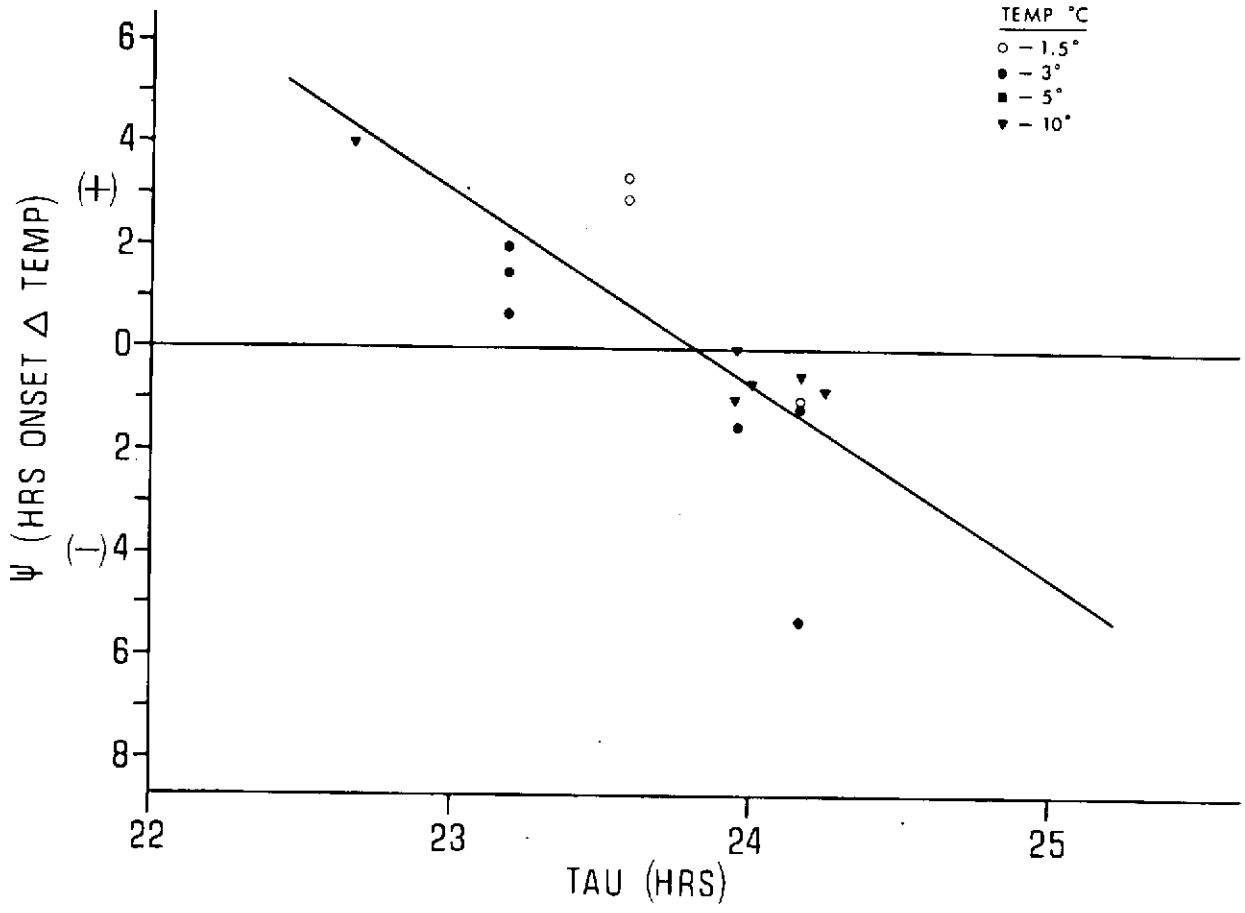


Figure 33. Relationship of the phase angle (ψ) of the circadian rhythm body temperature (τ) to onset of a temperature regimen, as a function of the free-running τ expressed following exposure to the temperature regimens. Experiments conducted in constant dark and constant temperature ($20^{\circ} \pm 0.1^{\circ}C$) except for programmed rises in temperature of six hours duration. Symbols denote average times of arousal from torpor (phase reference point).

phase angle to correlate with a shortening of τ_{FR}. In this sense the data are in agreement with properties exhibited in entrainment to light and generally support an oscillator model of the circadian system.

C. Discussion

Light and temperature are generally accepted as the dominant environmental factors which entrain circadian periods. While light appears universally effective as an entraining agent, only poikilotherms have been shown to entrain to temperature cycles (9, 10, 11). Clear evidence of entrainment of homeotherms to temperature has not been demonstrated. Mammals such as hamsters (12), flying squirrels (13), and the Rock Pocket Mouse, Perognathus intermedius (14) have been shown not to entrain to temperature cycles. Swade (15), on the other hand, concluded that temperature cycles could not be ignored as entraining agents for arctic rodents but that their effectiveness "was at best secondary and slight" compared to light cycles.

Perognathus longimembris spends the daylight hours in a burrow at depths ranging from as little as 1 cm to as much as a meter or more depending on time of day and time of year (1). The burrow is usually plugged with loose dirt. Trapping records indicate the P. longimembris is most abundant in trap lines in the first half of the night. We have observed no mice above ground in either the early evening or predawn periods. Kenagy (16) reports trapping only one P. longimembris in the twilight period with about 95% of trap yield occurring in the first 3/4 of the night. It is difficult to accept photoperiod as an ecologically effective zeitgeber for this species unless it is assumed that it is responsive to very low light intensities.

We exposed P. longimembris to four hour light pulses while defining a phase response curve to light (Figure 33). Intensities of 230 lux in many cases failed to illicit a response although light in excess of 930 lux produced pronounced phase shifts. Further there is no evidence that full moon nights

inhibit above ground activity. Accidental exposure to bright light would tend to reinforce nocturnal behavior but it is reasonable to assume that P. longimembris is relatively insensitive to the light intensities normally experienced between sunset and sunrise.

Explanations of how temperature can effectively entrain P. longimembris in nature are largely dependent upon the assumed behavior of the animal in its burrow. Data from burrow excavations and field behavior of P. longimembris by Kenagy (1, 16) permit some speculation on the way in which temperature could be an ecologically effective zeitgeber.

P. longimembris can occupy any part of the burrow system but during the spring and summer it typically chooses an ambient temperature of 26-30°C for resting. In the spring these temperatures occur in the top 10 cm of soil, and at 20-30 cm depth in the summer. In the summer, the mean burrow temperatures at 20-30 cm depth range between 26-30°C with a maximum daily fluctuation of 2.6-3.3°C. Peak burrow temperature occurs 2-3 hours after sunset. If the animal in Figure 31 remained at 20 cm depth and was subjected to these conditions, one would predict arousal from torpor to occur at about sunset with major above ground activity beginning between 10 and 12 PM. However, if the mouse occupied a shallower burrow depth (which it frequently does), then increases in burrow temperatures in excess of 3°C would be experienced earlier in the day resulting in above ground activity earlier in the night. These predictions are in reasonable agreement with above ground activity measured by live trapping.

However, Kenagy observed almost continuous subsurface activity in P. longimembris during the summer which suggests that the animal does not remain in one place (1). It is not clear to what extent the movement alters the thermal environment experienced. With regard to onset of above ground activity, A. French (Zool. Dept., UCLA, private communication) suggests that, since P. longimembris actively seeks zones of thermal neutrality, that it may move about the burrow as the burrow warms but be precluded from leaving by high burrow temperatures near the surface.

Near surface burrow temperature would not cool to a habitable level until after sunset (1).

However one chooses to interpret the data it is clear that P. longimembri can discriminate small changes in temperature and, in the laboratory, will entrain to temperature cycles of an amplitude shown to occur in their burrow. This may be the first demonstration of entrainment of a rhythm by temperature in a mammal. This observation suggests the importance of temperature as a zeitgeber for this species.

V. CHANGES IN PHASE OF THE CIRCADIAN RHYTHM OF BODY TEMPERATURE RESULTING FROM SINGLE EXPOSURES TO A ZEITGEBER

A. Methods

1. Phase Response to Light Stimuli. Data reported herein were obtained from two sets of experiments. The first experiments involved handling the mice during stimulus. In the second set of experiments the mice were not disturbed. No differences in experiment results were detected between the two protocols.

For the first set of experiments 24 mice previously used to study the stability of long term free-runs were selected. All were implanted with biotelemeters for monitoring changes in body temperature. Initially, the 24 were sorted into six groups. One animal in each group had a documented free-running period of less than 24 hours, one animal a period of greater than 24 hours, one animal a period close to 24 hours, and one animal an imperfectly defined period. (Period length did not appear to influence the results and in subsequent experiments animals were grouped indiscriminently.

The animals were held in constant dark (DD) for three weeks and the free-running period (τ_{FR}) measured. Time of arousal from torpor was chosen as the phase reference point (ϕ_R) and all exposures to light referenced to that event.

Exposure was effected by removing an individual animal from its monitoring cage in DD and placing it in a gallon jar with a sand and seed substrate. The jar with the mouse was placed on a laboratory table under fluorescent lights for 4 hours at 1160 lux. The mouse was then returned to its monitoring cage in DD and body temperature monitored for three weeks. It should be noted that this technique required that the animals be handled. As a consequence individuals that were torpid were aroused. In the second set of experiments 17 mice were added to the original group of 24. The protocol was repeated but modified to avoid disturbing the animals during stimulus (see III-A). In all, data were collected from 18 males and 23 females.

Phase shifts ($\Delta\phi$) were calculated from the "steady state" free runs (τ_{SS}) before and after stimulus. Tau's were calculated by a least squares fit of observed ϕ_R's. The reliability of ϕ_R for estimating τ_{SS} has been documented (2). In some cases, however, animals make "errors" and arouse either early or late. Our experiment protocol utilized ϕ_R to determine the approximate circadian time (CT) at which a light pulse was to be administered, and the reported CT was derived after the fact from the position of a curve fit to the ϕ_R's. If data are simply plotted with reference to time of arousal, the scatter of points obscures the shape of the curve. We interpret this observation as an illustration of an underlying circadian oscillator to which our observed endpoints are imperfectly coupled.

2. Phase Response to Temperature Stimuli. Experiment conditions were the same as described in IV-A. Eight male and twelve female adult mice were selected for study on the basis of the precision of the free-running rhythm of arousal from torpor demonstrated in earlier experiments, and their predilection to express torpor. All mice had been collected from the field and held in the laboratory for 12-18 months prior to this experiment. The experiment protocol consisted of administering three different kinds of temperature pulses at different circadian times to "free-running" animals

held in DD. The first kind of pulse consisted of a 6 hour rapid rise in temperature (10°C). Second a 10°C pulse was administered for only 1 hour. Thirdly the temperature pulse was administered with a slow rise time to reach a peak value of either 10°C six hours after initiation or 5°C three hours after initiation, after which the stimulus was removed. Except for programmed temperature excursions, cage air temperatures were held at 20° ± 0.1°C for all protocols. Data were analyzed as described above.

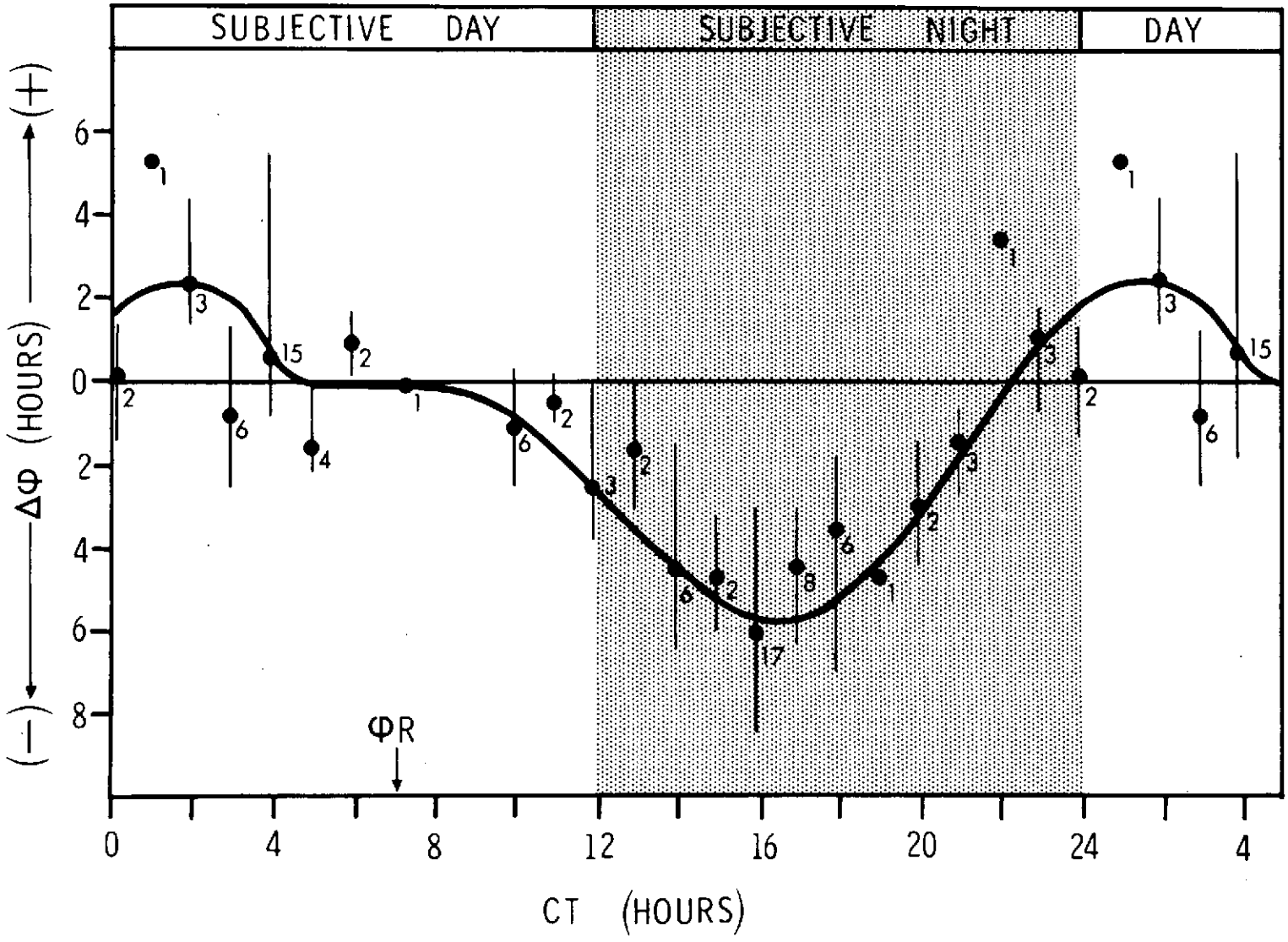
B. Results

1. Phase Response to Light Stimuli. In Figure 34 the observed changes in phase are plotted as a function of circadian time. The observed phase shift is plotted at the midpoint of the four hour stimulus. The time of all phase-shifts has been normalized to 24 hours by multiplying the observed time of the shift by $\frac{24 \text{ hours}}{\tau_{FR} \text{ hours}}$. Location of ϕ_R was estimated as described below.

Phase delays occur early in the subjective night and phase advances early in the subjective day. The data are more similar to Glaucomys than to Mesocricetus in that the refractory period is relatively short implying, in this case, that for about 18 hours of the circadian period the animal is capable of responding to a zeitgeber.

2. Phase Response to Temperature Stimuli. The phase response curve obtained from temperature pulses (Figure 35) was derived from a population of animals collected from the field. The range of observed values, therefore, is not surprising. CT 00 is equivalent to dawn of the first day of DD following entrainment to LD 12:12. We located the position of ϕ_R on the CT scale by the following reasoning. Arousal from torpor (ϕ_R) anticipates dark by 7 hours in an LD 12:12 regimen (this value was extrapolated from Figure 30). However, light surpresses activity and represents an unnatural circumstance to the nocturnal and fossorial P. longimembris. In DD the pattern of activity shows a major burst 4-6 hours after arousal

Figure 34. Changes in phase ($\Delta\phi$) of the circadian rhythm of body temperature in Perognathus longimembris resulting from single exposures to 4 hours of white light (930-1160 lux). Phase changes were determined from steady state free-running periods (τ_{SS}) expressed in constant dark at 20°C before and after stimulus. τ_{SS} were calculated by a least squares fit of observed times of arousal from torpor (ϕ_R) and τ_{SS} normalized to a value of 24 hours to correspond to the formalized 24 hours of circadian time (CT). Values within one half hour of the whole CT hour were grouped together to produce the average (dot) and range (bar). Numerals denote sample size. Data are plotted at midpoint of stimulus. Curve fit by eye. Cage air temperature was maintained at 20° ± 0.1°C.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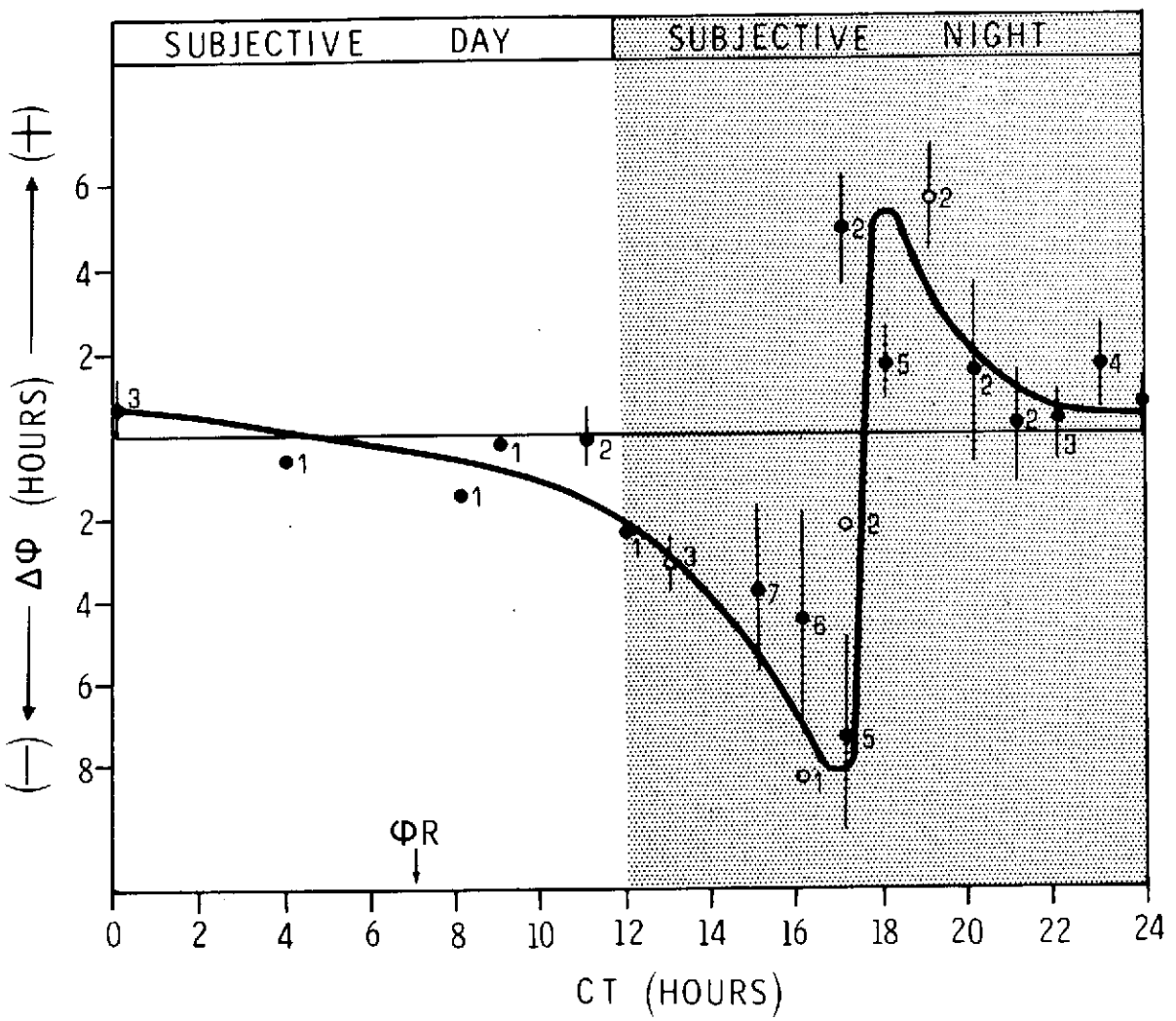


Figure 35. Changes in phase ($\Delta\phi$) of the circadian rhythm of body temperature in Perognathus longimembris resulting from single exposures to increases in air temperature from 20°C to 30°C. Phase changes were calculated from the steady state free-running period (τ_{SS}) expressed in constant dark at 20°C before and after stimulus. τ_{SS} were calculated by a least squares fit of observed times of arousal from torpor (ϕ_R) and τ_{SS} normalized to 24 hours to correspond to the formalized 24 hours of circadian time (CT). Data within one half hour of the whole CT hour were grouped together to produce an average (circle or dot) and range (bar). Solid dots are six hour, and open circles one hour duration temperature pulses. Data are plotted at onset of stimulus. Numerals indicate sample size. Curve fit by eye.

which we believe corresponds to onset of above ground activity. Since P. longimembris is not taken in traps in twilight hours but is abundant in the first half of the night we reasoned that the average ϕ_R anticipated above ground activity by six hours and sunset by five hours.

As in the case of Figure 34, the phase response curve was derived from a population of animals and all values within 1/2 hour of the whole hour were grouped together. The rather sudden change in response from phase advance to delay is not unlike those reported from light pulses to Kalanchoe (17), Drosophila (18), and Mesocricetus (12).

Data collected from one hour temperature pulses are also plotted in Figure 35. Since the amplitude of response is similar to that expressed by animals exposed to 6 hour pulses it is reasonable to conclude that the mice are responding to the temperature rise rather than the duration of the pulse. All values in Figure 34 therefore are plotted with reference to onset of stimulus.

If P. longimembris only responds to rapid rises in temperature, then the ecological significance of the data reported would be limited. Consequently animals were exposed to slow rise times in which temperature uniformly increased. Three animals were exposed to a slow temperature rise from 20°C to 30°C over a six hour period, and two mice were exposed to a slow rise from 20°C to 25°C over a three hour period. Both of the mice exposed at CT 16 to a 5°C slow rise showed a phase delay of -3.5 hours. All three animals exposed at CT 16 to the 10°C slow rise responded with phase delays of -4.7, -7.3, and -5.8 hours respectively. These data indicate that P. longimembris will respond to slow rises in temperature comparable to what could be anticipated to occur in shallow burrows, and further, that the amplitude of the phase shift is comparable to that observed for rapid rise times. We are therefore justified in considering the ecological implications of the data.

3. Interaction Between Light and Temperature Acting as a Zeitgeber. The phase response curves provided by light and temperature stimuli are similar in both the amplitude and slope of the delay portion of the curves (Figure 36). This observation gave rise to the question of whether light stimuli would dominate temperature stimuli as it does in other organisms. A single pilot experiment was undertaken which showed that temperature and light stimuli when applied together were additive (Figure 37). This experiment has not been repeated and other interpretation are possible. The implication, however, was that light and temperature are mutually effective zeitgebers for Perognathus longimembris.

C. Discussion

The fact that organisms not only possess an accurate time sense but that the sense can be adjusted to local time by discrete advances or delays in response to environmental stimuli (primarily photoperiod) has been extensively documented. The phenomenon of resetting of the biological "clock" in response to environmental stimuli (zeitgebers) is the mechanism by which the biological "clock" may be driven at periods different from its natural frequency (i. e. entrainment by a zeitgeber). The "Bünning Hypothesis" postulates that an organism is not equally sensitive to environmental stimuli at all times of the day, and that duration of light or dark is the primary stimulus involved in keeping an organism in synchrony with seasonal changes. The phase response curve has been a useful tool in testing that premise. The curve is generally obtained by exposing an organism to a stimulus for a short interval at different parts of its circadian period and noting the degree to which the phase of the biological clock is advanced or delayed.

Conversely, an environmental stimuli suspected of being a zeitgeber may be tested by exposing an organism to it for short intervals to determine if a phase shift will occur. The test, however, is of limited use since there are at least two classes of zeitgebers. Strong entraining agents, such

as light, are those in which a single exposure to the stimuli can result in a shift in phase of the biological clock. Weak entraining agents, on the other hand, must be repeatedly administrated at regular intervals to result in entrainment. Light has been established as the most effective and most ubiquitous zeitgeber but other stimuli such as cyclic changes in temperature, sound, hydrostatic, and air pressure have also been shown to be entraining agents.

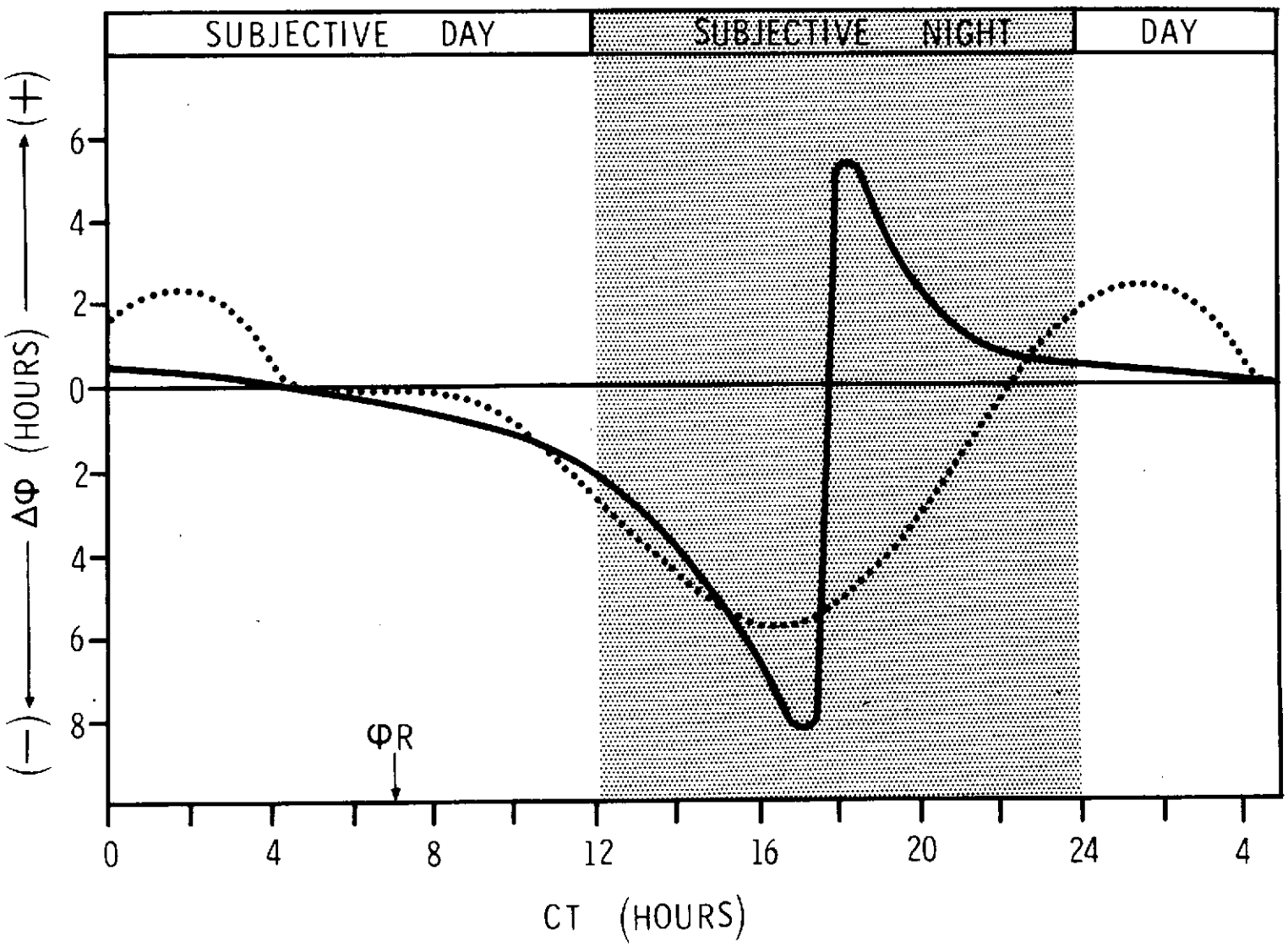
With respect to preparation for Skylab Experiment S-071, with all caveats acknowledged, we assumed that a phase-response curve derived from light stimuli would be a meaningful estimate of the range of possible response of the pocket mouse circadian system to a wide range of potential zeitgebers. More conservatively it was a logical point of departure in discussing the possible effects of isolated perturbations to Experiment S-071. We recognized that there was no one phase response curve for an organism, but we were equally convinced that, for the pocket mouse, the general character of the curve would be retained irrespective of the kind of zeitgeber.

A phase response curve to light stimuli was defined and determined to be generally characteristic of nocturnal animals, but more similar to the crepuscular flying squirrel than the nocturnal hamster. A phase response curve to temperature stimuli was similar to that defined for light except that the transition from phase delay to phase advance occurred more abruptly and midway in the subjective night.

The similarities in the two response curves led to speculation as to whether light as a zeitgeber would dominate temperature in pocket mice. Results from a single experiment implied that light did not dominate and that light and temperature stimuli administered together produced an additive effect.

Perognathus longimembris entrains to periodic rises in ambient temperature of a magnitude similar to that experienced in its burrow (2-10^{o}C). The phase response curve examined in the perspective of the animal's

Figure 36. Comparison of two phase response curves of the circadian rhythm of body temperature in the little pocket mouse, Perognathus longimembris. Data were derived from single exposure to light pulses (dotted line) and temperature pulses (solid line) during a free-running condition in constant dark and constant temperature ($20^{\circ} \pm 0.1^{\circ}C$). Refer to captions in Figures 34 and 35.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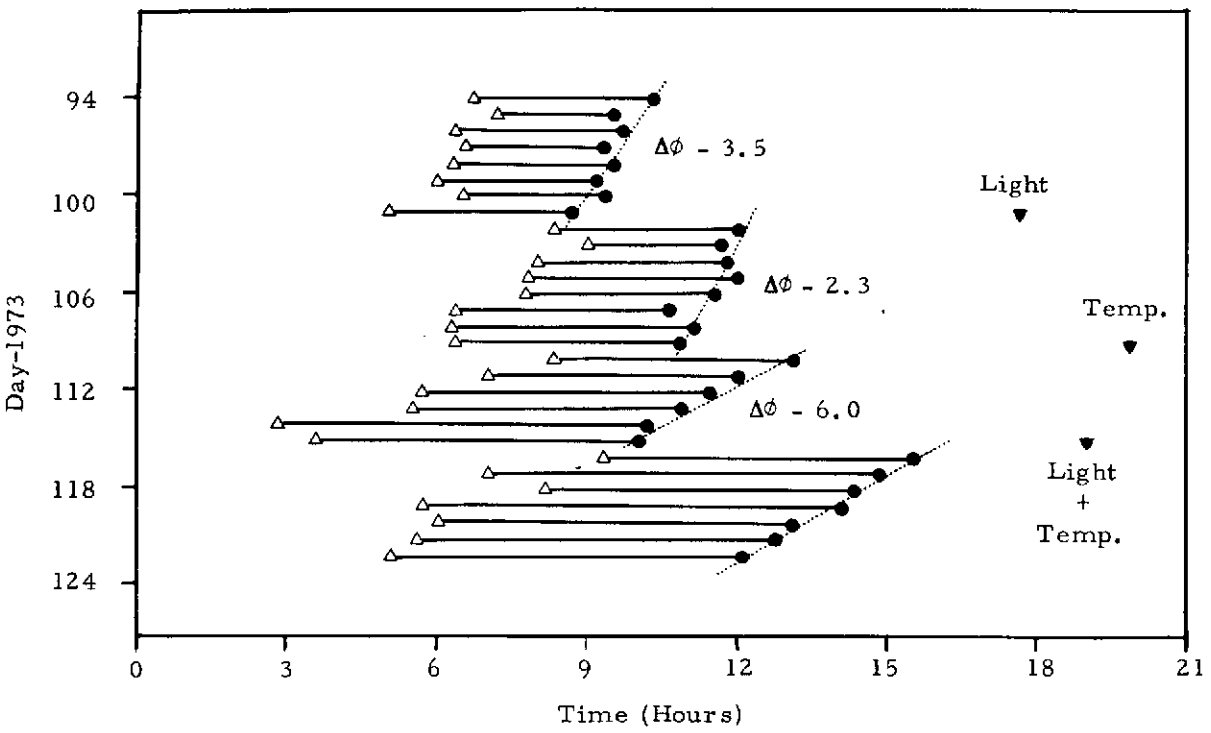

Figure 37. An example of the additive effect on phase response produced by simultaneous stimulation by light and temperature. A light stimulus (930 lux) of four hours duration was administered from a mercury light source filtered through a saturated solution of copper sulphate. Temperature increases of 10°C were administered for six hour periods. Time of entry (open triangle) and arousal (dot) from torpor are plotted on successive days. Except for periods of stimulation the mouse was maintained in constant dark and constant temperature (20° ± 0.1°C). The response to the combined stimulus is approximately equal to the sum of the stimuli administered separately.

ecology supports the premise that exposure to light may be unnecessary to maintain the animal's above ground activity in synchrony with the dark of night.

VI. GENERAL DISCUSSION

A. Properties of the Circadian Rhythm of Body Temperature in Perognathus longimembris

Characteristics of the free-running circadian period of P. longimembris are similar to those of other nocturnal mammals reported in the literature. The "free-running" circadian period (τ_{FR}) by definition, is the period expressed by an organism held in a constant environment shielded from entraining agents (zeitgebers). It is not a fixed characteristic of individual animals but is limited to a narrow range of values (nominally 23-25 hours). The precise τ_{FR} expressed at any time is a function of the environment immediately preceding the steady state free-run and the physiological state of the animal. Transients almost always precede attainment of a new steady state. The phase of a free-running period may be shifted by a single exposure to an entraining stimulus and the subsequent τ_{FR} is characteristically different in length but does not necessarily degrade in precision. These general traits have been verified for Perognathus longimembris while housed both in laboratory monitoring equipment and space experiment hardware.

The persistence of a given τ_{FR} was shown to be good over periods of as long as a year for most animals. Some mice, however, slowly drifted from short τ_{FR} to long τ_{FR} and back again over several week's time. The pattern was reoccurring. The overall impression was that the τ_{FR}, while persistent, is very labile in this species.

Entrainment of the circadian rhythm of body temperature, likewise, was typical of other nocturnal mammals and generally tended to follow the "rules" governing circadian behavior.

Certain of the properties of circadian rhythms exhibited by Perognathus longimembris are worthy of note as a point of departure for considering analagous "models."

1. The presence of an ultradian (< 24 hours) oscillator is suggested from analysis of daily records of both body temperature and animal activity. Additional evidence for the presence of an ultradian oscillator is found in deviations in times of arousal from torpor from times predicted by the circadian period; times of partial arousals from torpor; times of arousal from multiple torpors occurring in the same day; and consistent alternate patterns of arousal over an extended time. All of these examples have in common, variations in time of occurrence which tend to be whole multiples of the period of the ultradian oscillation estimated for a given animal.

2. Temperature and light appear to be equally effective as entraining agents (zeitgebers). The properties of response to light and temperature appear somewhat different. Degree of response to light is dependent (within limits) upon intensity and duration of stimulus, while response to temperature appears to depend upon the stimulus exceeding a threshold to produce a total ungraded response.

3. The circadian period of body temperature characteristically shortens in animals expressing torpor which are maintained at low ambient temperature. This is an unusual response reported in only one other mammal (Glis glis).

4. Frequently changes in the circadian period are noted following a light or temperature stimulus for which no phase shift occurs. Occasionally the circadian period will change abruptly and apparently spontaneously in the absence of zeitgebers.

5. There is a tendency over a period of several months for the free-running circadian period of body temperature to slowly drift from less than 24 hours to greater than 24 hours and back again.

B. Speculations on Frequency Demultiplication as a Model for Circadian Rhythms in Pocket Mice

That part of the circadian system that we have studied may be likened to a form of frequency demultiplication in which one oscillator (circadian) counts off a whole number of another's beats (ultradian) for each one of its own. Thus the ultradian oscillation apparent in animal activity records has a period of approximately 90 minutes. The circadian oscillator "counts" or registers 18 beats of the ultradian oscillator to generate a once a day event such as arousal from torpor. Whether the ultradian "generator" or the circadian "counter" is more sensitive to environmental stimuli is uncertain. However, many observed phenomenon are conveniently explained by assuming some degree of autonomy between the two oscillators. Observed characteristics of circadian phenomenon listed above are not inconsistent with such a model.

In concept the model is not new. What is new is a mammalian circadian system in which the many premises can be tested by manipulation with apparently mutually effective zeitgebers (light and temperature). The statement is perhaps unnecessary but it should be clearly understood that the model is probably simplistic in terms of the way in which the biological system probably operates. The model, however, has some predictive value and appears a useful concept for guiding future research.

C. Speculations on a Positively Skewed Circadian Oscillator as a Model for Circadian Rhythms in Pocket Mice

Aschoff et al. (19) proposed a theoretical model of circadian systems of birds and men based upon the mathematical models of Wever (20). The parameters of activity (α) and rest (ρ) were used to test the model. If torpor in the pocket mouse is considered as a special case of rest, the mode described by Aschoff et al. can well account for our observations.

1. The Model. Figure 38 is a representation of the model as applied to the circadian system of pocket mice. The model assumes a positively skewed circadian oscillation. Activity and rest (torpor in the case of the pocket mouse) are partitioned relative to the position or level of a threshold representative of a particular physiological state. Intersections of the threshold with the circadian oscillation signal entry and arousal from torpor. Observed variations in entry or arousal from torpor could be explained by hysteresis in either or both the threshold and the oscillator.

The deviations in observed times of arousal from torpor from the predicted time based on the estimated circadian period are discussed in V-A above. Those observations led to the conclusion that the time of arousal was imperfectly coupled to an underlying circadian oscillator. The shape of the circadian oscillation depicted in Figure 38 is derived as a best fit for observed data and may in truth have little relationship to the shape of the "real" circadian pacemaker. Aschoff et al. refer to this kind of curve as a "form of function."

The relative slopes of the ascending and descending portion of the "form of function" may be estimated from the standard deviations of times of entry and arousal from torpor. Mean standard deviations for entry and arousal were calculated from 24 animals during three experiments totaling 69 days in duration (1656 mouse days). The average standard deviation for entry was 1.43 hours, and for arousal 0.86 hours. The ascending slope, therefore, is approximately 60% greater than the descending slope resulting in a positive skew.

2. Threshold Level. Data suggest that the level of the threshold is very labile in P. longimembris. This observation may relate to the fact that P. longimembris appears to hibernate during the winter. Very long torpors of up to 3 days duration are typically expressed by animals held in constant dark, with ample food, and at low ambient temperatures (5-10^{o}C). In the field in the summer, however, Kenagy (16) has observed relatively continuous activity with no well defined rest period.

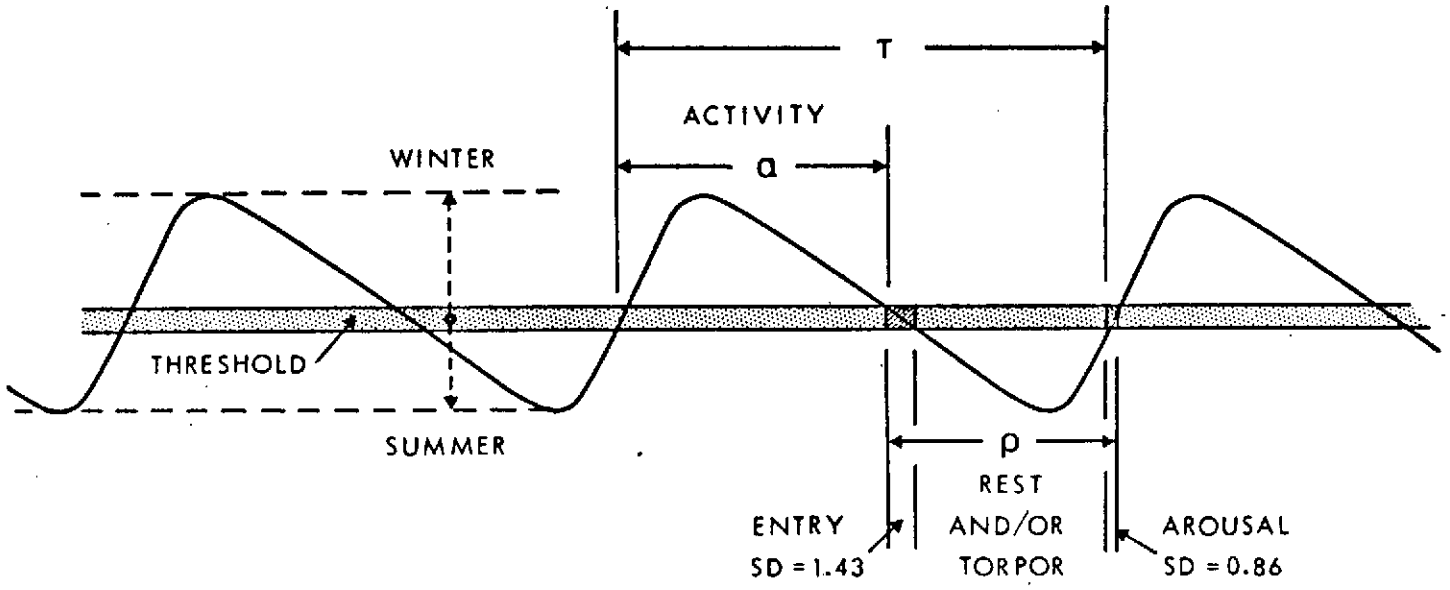

Figure 38. A proposed model for the circadian system of the Little Pocket Mouse, Perognathus longimembris. (After Aschoff et al. Ref. 19.)

These observations can be explained if the threshold is indeed considered a measure of physiological state subjected to strong displacement upward in the winter and downward in the summer. During the winter the threshold reaches an upper limit such that activity, if expressed at all, is restricted to a very small part of the day (2-4 hours). During the summer the threshold drops to a lower limit and a well defined rest period is very short. The limits are determined by the amplitude of the circadian oscillation which continues to provide a once a day signal to the system in both winter and summer.

3. Annual Cycles. Some speculation is warranted as to whether the seasonal placement of the threshold level is governed by an endogenous annual rhythm or by exogenous factors. In the laboratory torpors representative of a winter physiology are observed at all times of the year. Animals not expressing torpor in the colony begin to go torpid when transferred to the laboratory and are maintained in constant dark, constant temperature and with an abundance of food. The transition from no torpor to torpor, however, is not accomplished in a single step. Rather, there is typically a pattern of sequential torpors which gradually increase in duration not unlike the "test drops" reported in animals preparing for hibernation (21).

Initiation of regular torpors in P. longimembris are characterized by three steps. First, "test drops" occur over a period of several days in which during rest the body temperature lowers for an hour or two but remains significantly above ambient temperature. Second, body temperature lowers to within a degree or so of ambient followed by daily sequential increases in duration (Figure 39). Third the duration of torpor stabilizes and appears to encompass the total "rest" period. The entire transition usually occurs within 1-3 weeks. However, very rapid transitions from torpid to non-torpid behavior have been observed associated with sudden rises in ambient temperature, and abrupt transitions from short torpor to very long torpor can be induced by removal of food. Ambient temperature per se does not seem to govern the duration of torpor.

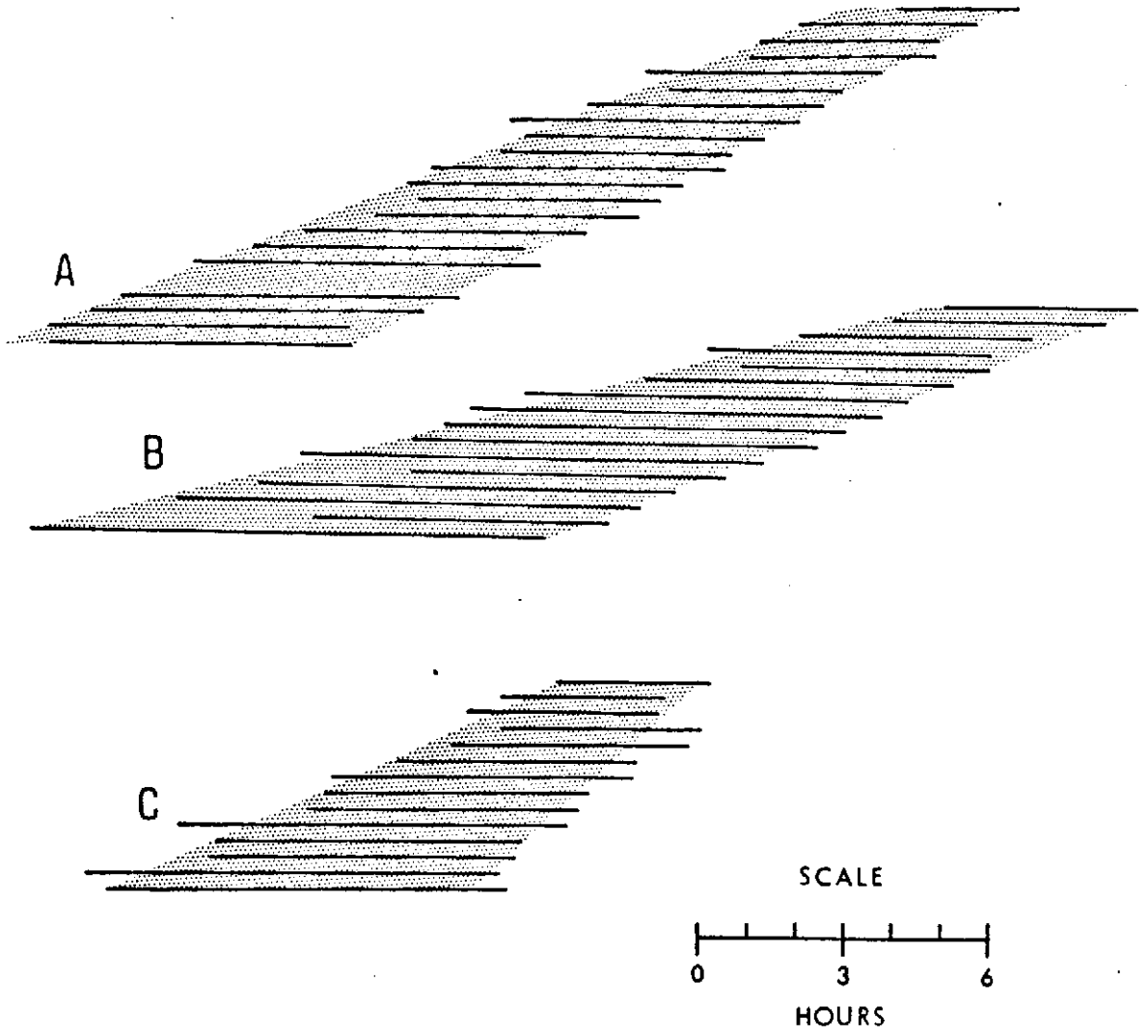

Figure 39. Three examples of patterns of increasing duration of torpor in Perognathus longimembris during the transition from non-torpid to torpid behavior. Bars indicate time in torpor on successive days.

It would appear that the threshold level normally moves toward the summer or winter limits in response to environmental cues, but that in times of stress the mouse can override the trend and place the threshold at the level of maximum survival value. It is tempting on the basis of this discussion to conclude that the annual cycles reflected in patterns of torpor are driven by exogenous factors.

4. Role of an Ultradian Oscillator. There is some evidence for the presence of an ultradian oscillator being implicated with patterns of animal activity (Tables IV, V, Figure 19). Other evidence for the presence of an ultradian oscillator is presented in paragraph IV-A above. A circadian system modeled after some form of frequency demodulation is discussed in paragraph IV-B above. There is some evidence that "errors" in times of entry and arousal from torpor are whole multiples of a 90 minute interval indicative of the ultradian oscillator. The 90 minute cycle appears to persist during both activity and rest. During the active period it appears to stimulate bursts of activity. During rest it appears ineffective but its presence is indicated by occasional false arousals and wakening in sychrony with the 90 minute cycle.

If it is assumed that the 90 minute cycle represents an "agitation" stimulus, then this 90 minute rhythm superimposed on the threshold could modulate the exact time of arousal. In other words, the first occurrance of a 90 minute signal within the active period determined by the circadian oscillation should cause arousal. Whether arousal occurs or not does not impede the progress of the daily cycle.

5. Conclusion. Given these observations and assumptions it would seem that the intersection of a given threshold level (with some hysteresis) with the circadian oscillation (also with some hysteresis) modulated by 90 minute "gates" should circumscribe an area which reflects the observed variations in time of entry and arousal from torpor. In the light of this model relatively large standard deviations of the free running circadian period of body temperature are to be expected in Perognathus longimembris.

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PART IV

Appendices

APPENDIX A

Selection of Species and Development of Animal Activity and Heart Rate Monitor

Extracted from Report of Progress

20 May 1968 through 31 March 1969

INTRODUCTION

Body temperature as a function of time is the primary datum required for the study of the stability of the circadian system of pocket mice in space. This is the parameter which has been intensively studied in the laboratory; documented in the literature[1]; and ultimately led to the submission and acceptance of an orbital experiment (MSFEB S-071) scheduled for implementation during the Apollo Applications Program. Recent advances in biotelemetry indicate that it is now feasible to monitor not only body temperature but also animal movement and heart rate from a single telemeter implant. Development of the monitoring technique has proceeded so successfully that monitoring of all three biological parameters is being seriously considered for Experiment S-071 and related experiments in deep space. The scientific merit of these experiments increases appreciably with the number of biological parameters that can be monitored simultaneously. The approach initiated under this contract, therefore, emphasized (1) experiments which monitor as many biological parameters as prove meaningful and practical to measure remotely, and (2) experiments of long duration to examine the stability and interrelationships of the biological parameters selected for study.

At the end of the first quarter effort it became necessary to redirect the program to more urgent problems associated with the definition of a short term (6 week) experiment in space. The research is anticipated to provide data which will permit selection of a specific experiment plan from the present options being considered for experiment MSFEB S-071, "A Study of the Circadian Periodicity of Pocket Mice." The options being considered are concerned with manipulation of the experimental animals pre-launch and the monitoring of the circadian periodicity of heart rate. None of the options require significant changes to the experiment hardware defined for MSFEB S-071 under Contract NAS2-5093.

At this point in time, and in the context of the objectives of Experiment S-071, several alternative experimental designs are feasible.

1. Monitoring of body temperature alone in Perognathus longimembris.

2. Monitoring of body temperature and animal movement in Perognathus longimembris.

3. Monitoring of body temperature, heart rate, and animal movement in a larger species of pocket mouse such as Perognathus parvus.

Selection of an experiment option is dependent upon securing additional experimental data to:

1. Compare qualitatively and quantitatively the data derived from gross animal movement and "running wheel" behavior with regard to its usefulness for the study of circadian periodicity in several species of pocket mice.

2. Verification of the reliability of the Northrop-developed heart rate telemeter including the methods of implant and tolerance of animals with this kind of implant to launch dynamics.

3. Analysis of the coupling between the circadian periods of heart rate, body temperature and activity to (a) justify simultaneous measurement of three parameters and (b) develop new data reduction programs as needed for support of the space flight experiment.

4. Studies to demonstrate (a) the stability of the circadian system in pocket mice over extended periods of time (nominally 90 days) and at different times of the year; and (b) studies of the "after-effects" phenomenon of animals maintained under abnormal photoperiod regimes.

This report summarizes activities to date which contribute to the objectives summarized above.

METHODS AND MATERIALS

Our study of biological periodicity requires that the experimental subjects be isolated in closely controlled environments and that the subjects be undisturbed by the monitoring procedures. The mammalian studies utilize a small telemeter which is implanted in the abdominal cavity; a receiving antenna wound around a plastic cage containing the animal; and a simple preamplifier. The isolated cages and preamplifier are in turn located in a constant temperature room under a controlled photoperiod. External to the constant temperature room the signal from the preamplifier is received and processed in an automated data acquisition system. Selection of parameters for measurement and all adjustments, modifications and repairs to the data system can be accomplished without disturbing the experimental animals or their controlled environment. Some of the mammal cages contain running wheels, revolutions of which are counted and recorded via the data acquisition system.

Biotelemeters

Two types of implantable telemeters are being used in the current studies. The first is the "standard" body temperature transmitter from which is derived also the frequency of animal movements. The second kind of telemeter is in the process of evaluation and provides EKG signals in addition to body temperature and animal movement data.

The temperature telemeter utilizes a conventional inductive capacitance oscillator circuit with a thermistor serving as a variable resistance. The unit encapsulated with a parafin/beeswax mixture weighs less than 1 gram with a useful life of approximately 250 days. This circuit was modified by adding an amplifier stage and two electrodes which served to frequency modulate the temperature pulse frequency with EKG signals. Prior to this contract a prototype of the EKG telemeter was implanted in a Rhesus monkey and it was possible to concurrently monitor body temperature, EKG, animal movement, and under some circumstances, respiratory rate for as long as three months.

The prototype used for the monkey study was subsequently miniaturized by reducing the amount of encapsulant, more effectively packing the components, and substituting a smaller battery. This smaller telemeter was implanted in white mice with encouraging results and as a consequence was used for pocket mouse studies.

Animal Movement Monitor

Both EKG and body temperature telemeters implanted within the abdominal cavity of rodents were used to monitor gross activity. "Gross activity" is defined as voluntary changes in body orientation resulting from movements associated with life processes in the normal sequences of events in the rest-activity cycle. This definition is presented to differentiate gross activity from the specific organized behavior of wheel running.

The radiant energy of the telemeter as measured by the receiving antennae characteristically changes within the antenna system as a function of the transmitter orientation. The amplitude variation of the signal is diode detected and filtered. This signal is amplified and fed to a Schmitt trigger which provides a standard pulse output each time the animal moves above a certain threshold. These pulses are integrated and fed to a voltage controlled oscillator, which produces a frequency output proportional to gross activity. The pulses are stored and the frequency is sampled and recorded via the frequency monitoring system every 10 minutes. VCO's are reset to a specific base frequency after they have been sampled. The threshold of each activity monitoring unit is adjusted so that variation in signal strength associated with respiratory movements of the animal are not recorded as activity.

Wheel Running Monitor

Each rodent chamber has a running wheel which can be monitored via a stationary magnetically operated reed switch and a magnet attached on the axle of the wheel. This detector feeds a circuit similar to that already

discussed in conjunction with gross activity. The end result is a frequency value which is directly proportional to wheel revolutions.

Cockroach Activity Monitor

A cockroach is housed in a plastic running wheel with access to food and water on one stationary side of the enclosure. Movement of the "wheel" is detected via a reed switch and magnet. The pulses are detected and recorded in the same manner as wheel running in rodents. This study was discontinued before meaningful biological data were collected.

Data Acquisition System

Prior to initiation of the contract the data acquisition system had a capacity of 25 channels of recorded data. Sixteen channels were assigned to monitoring body temperature and eight channels for experimental and developmental research leading to routine monitoring of gross activity, wheel running and EKG.

Upon initiation of the contract the inovations for monitoring gross activity, wheel running and EKG were integrated into the data acquisition system in a manner which permits monitoring any of these parameters on a selective basis from any of the 16 available mammal cages. Cockroach activity monitoring was not integrated into the data system since this phase of the program has been postponed on advice from the contract monitor.

Theoretically 62 channels of mammal data can be taken continuously at a choice of sampling rates. It is possible to select either continuous analog data (via dual pen analog recorder), sequential analog data at a rate of every 2 minutes (via 12 point recorder) or sequentially printed out at any rate desired (usually 10 minutes, via the scanner and digital printout system). Continuous individual inputs to all three systems are possible.

Animal Species

One constraint on the use of a single telemeter which will monitor heart rate, body temperature, and animal movement simultaneously is that it is slightly larger than the telemeter used to monitor body temperature and animal activity.

Since the species (Perognathus longimembris) originally proposed for Experiment S-071 and related deep space missions weighs only 10 grams, any increase in telemeter size is undesirable. There are, however, several other species of pocket mice which also have well documented circadian periods of body temperature but range in size from 15-35 grams body weight. As the body weight increases, the metabolic rate decreases and as a consequence, a 20 g species can be exchanged for the 10 g P. longimembris with negligible penalty on existing hardware concepts and configurations[2,3]. In other words, utilizing a larger species of Perognathus may permit monitoring three separate biological parameters; in no way compromises the scientific merit of the initially proposed experiment (in fact, the scientific merit improves); and does not require redesign of life support systems concepts.

As a consequence animal species used in this study included the Little Pocket Mouse (Perognathus longimembris), the Great Basin Pocket Mouse (P. parvus), the Long Tailed Pocket Mouse (P. formosus), and the White Mouse (Mus musculus).

RESULTS

Evaluation of a Proposed Method of Monitoring Animal Movement

The time of onset of activity has been demonstrated as a definitive marker for the study of circadian periodicity in small animals. Classically this kind of study is conducted with tilt cages or more commonly with running wheels. Neither of these approaches are practical for space experiments.

We propose to monitor animal movement by scoring the number of changes in signal strength resulting from changes in orientation between coils of an implanted telemeter and the receiving antennae. As a consequence, there will be a tendency to produce continuous data in contrast to the intermittent data characteristic of running wheels. Evaluation of the proposed monitoring method was undertaken in two steps: First, a comparison of data collected simultaneously from running wheels and from changes in telemeter signal strength; and second, determination of circadian period derived from changes in signal strength in the absence of running wheels, which in turn was compared with the circadian period of body temperature determined over the same study period.

Results representative of the data collected during the first phase is presented for Perognathus longimembris (wt ~ 10 grams) in Figure 1; P. formosus (wt ~ 20 grams) in Figure 2; and P. parvus (wt ~ 20 grams) in Figure 3. The data are for single days during the initial portion of a "free-run" in constant darkness at 21° C ambient temperature. Running wheels were available in all cages but were apparently not used by P. longimembris and P. formosus. The correlation between running wheel behavior and gross activity for P. parvus is striking.

Results of representative data collected during the second phase is presented for Perognathus parvus in Figure 4 and for several species in Table I.

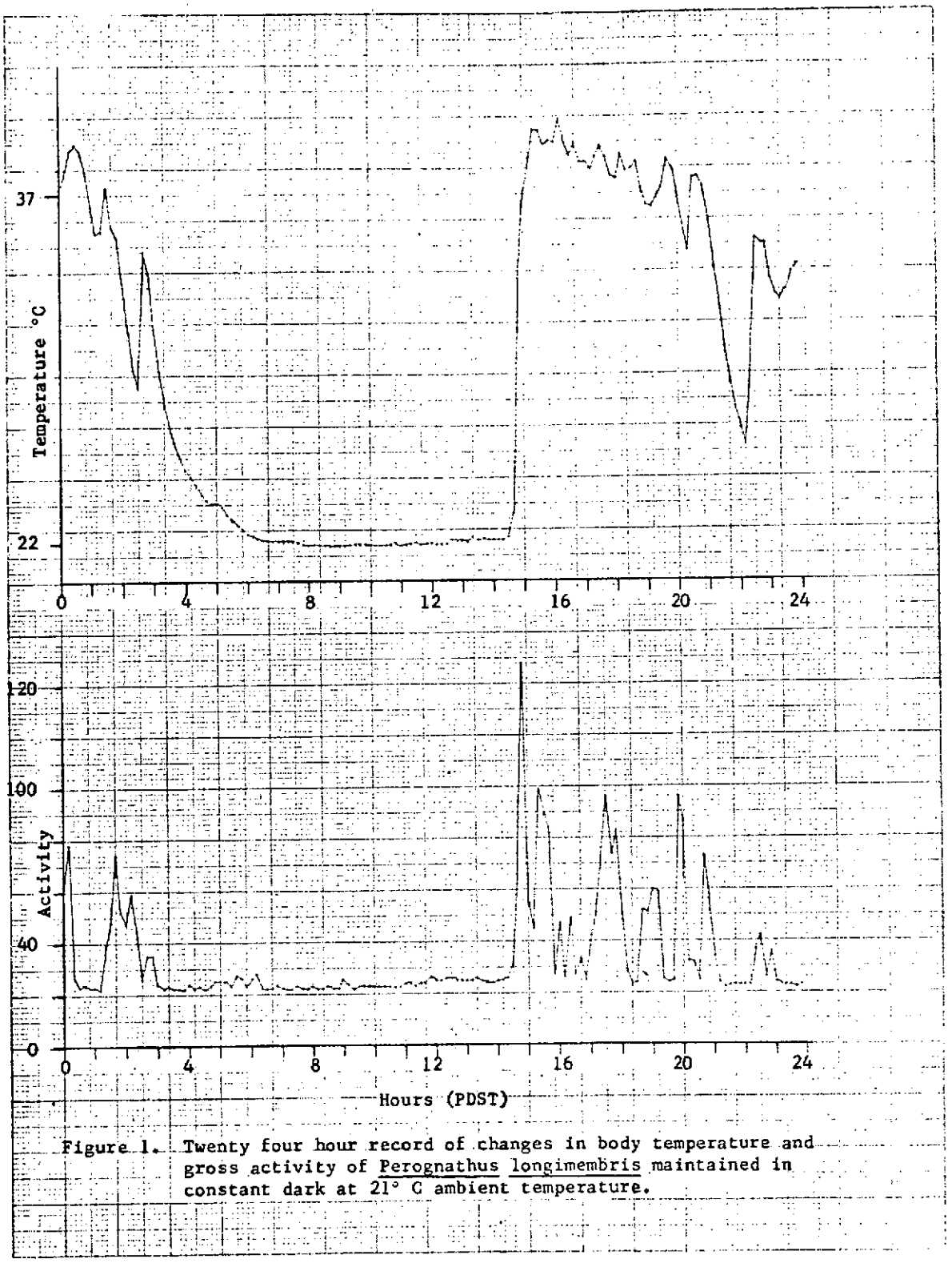

Figure 1. Twenty four hour record of changes in body temperature and gross activity of Perognathus longimembris maintained in constant dark at 21° C ambient temperature.

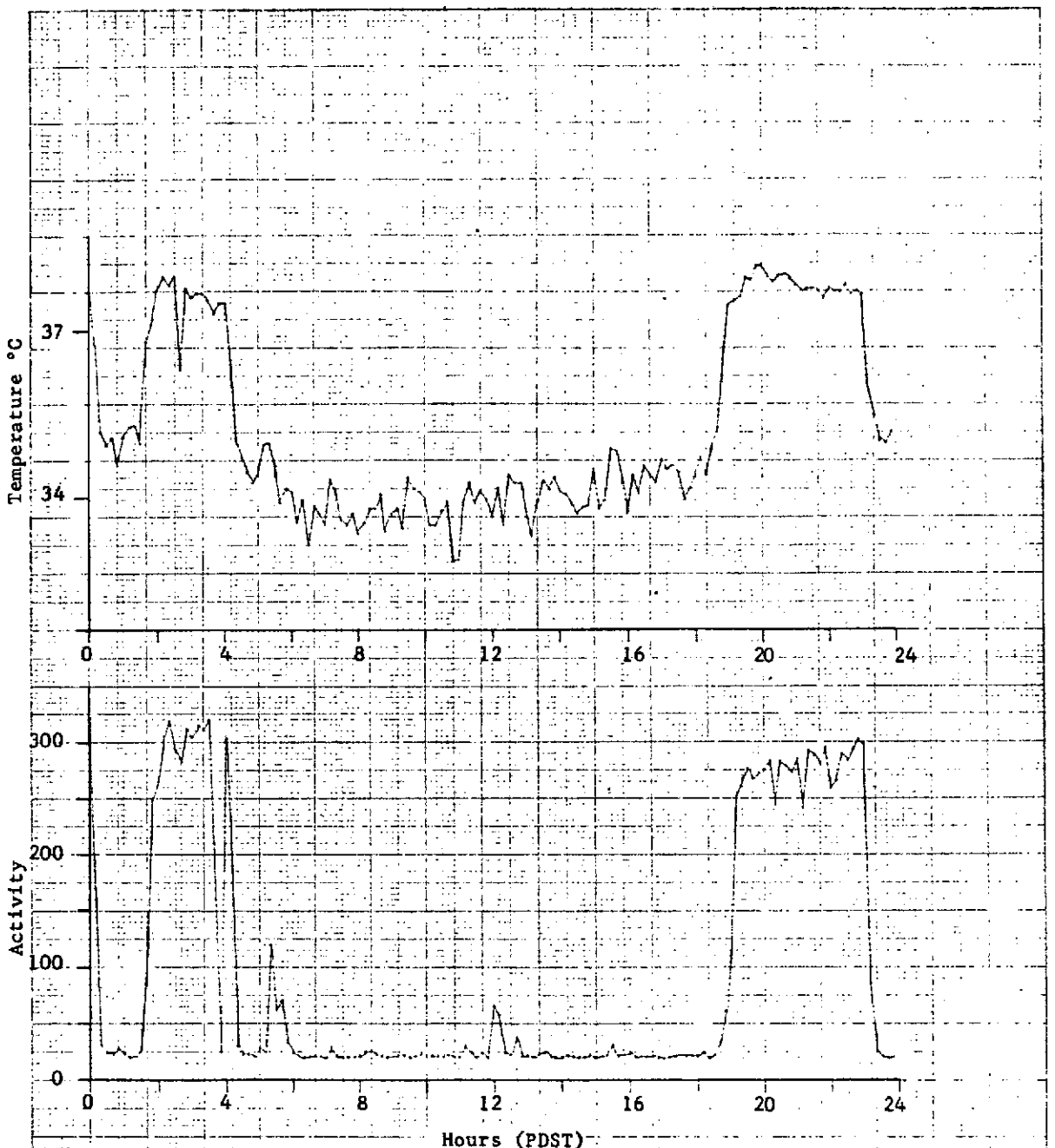

Figure 2. Twenty four hour record of changes in body temperature and gross activity of Perognathus formosus maintained in constant dark at 21° C ambient temperature.

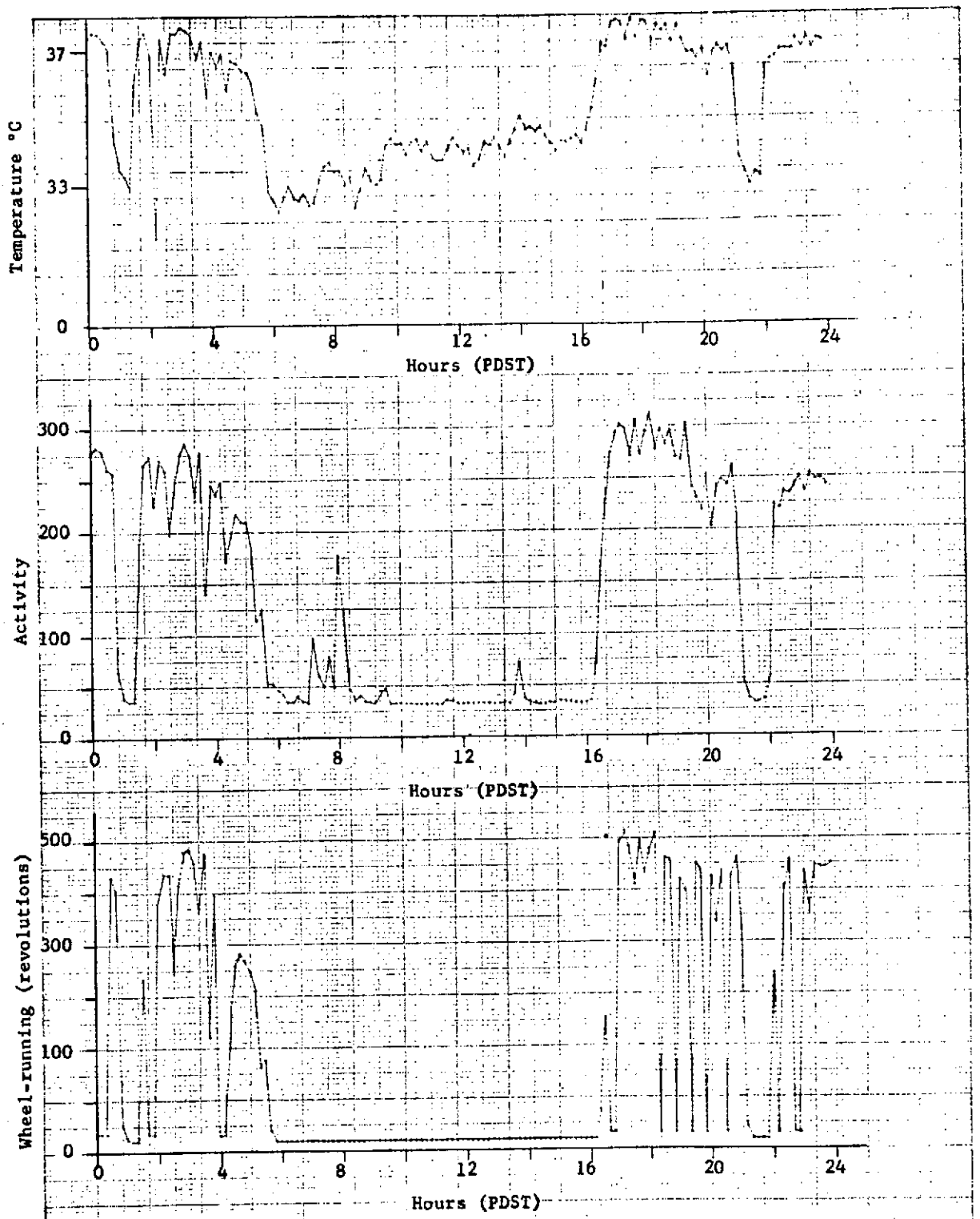

Figure 3. Twenty four hour record of changes in body temperature, gross activity, and wheel running behavior of Perognathus parvus maintained in constant dark at 21° C ambient temperature.

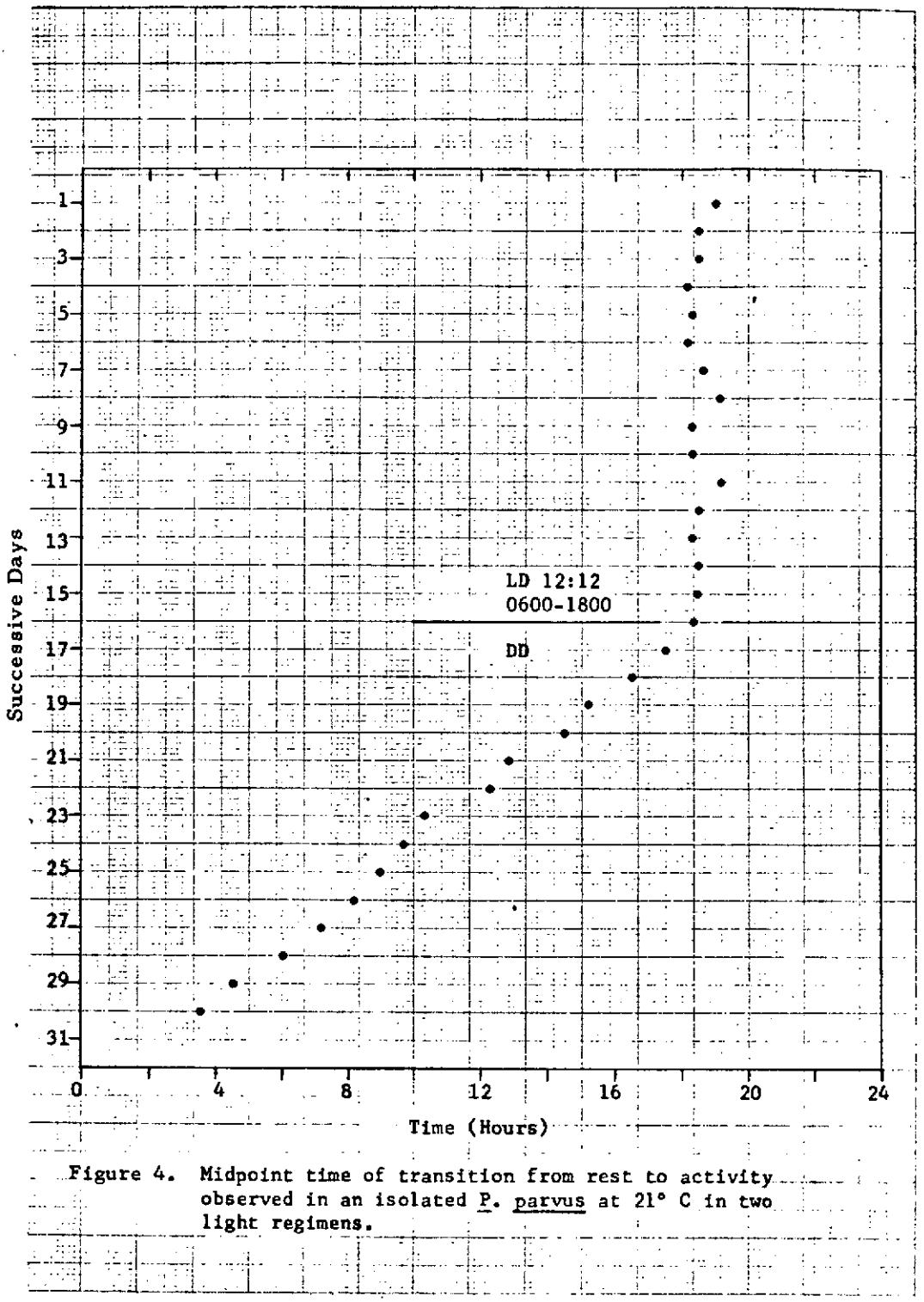

Figure 4. Midpoint time of transition from rest to activity observed in an isolated P. parvus at 21° C in two light regimens.

TABLE I

Circadian Periods (τ) of Pocket Mice Derived by Autocorrelation from Body Temperature and Activity Data of the Same Individual Maintained under Different Photoperiods at 21° C.

Species	Photoperiod	Days	τ (Hours)	
			Body Temp	Activity
Perognathus longimembris	LD	1-20	24.04 $\pm$ 0.09	23.59 $\pm$ 1.12
	DD*	21-34	23.88 $\pm$ 0.19**	23.82 $\pm$ 0.37
	DD	35-46	24.07 $\pm$ 0.13	24.21 $\pm$ 0.49
Perognathus formosus	LD	1-20	24.01 $\pm$ 0.06	24.06 $\pm$ 0.12
	DD*	21-34	23.96 $\pm$ 0.09	23.91 $\pm$ 0.24
	DD	35-46	24.21 $\pm$ 0.07	24.23 $\pm$ 0.51
Perognathus parvus	LD	1-20	24.02 $\pm$ 0.04	24.01 $\pm$ 0.10
	DD*	21-34	22.95 $\pm$ 0.85	22.94 $\pm$ 0.09
	DD	35-46	22.90 $\pm$ 0.51	22.93 $\pm$ 0.30

* The experiment was intended to be run in total darkness. Pinhole light leaks resulting from refrigeration maintenance were discovered following completion of the experiments. This may explain why neither P. longimembris or P. formosus appear to "free-run."

** A τ of 23.97 $\pm$ 0.14 was determined from this same data but was derived by analysis of the mid-point of arousal from torpor. The close agreement of the two values tends to validate autocorrelation analysis of this kind of data.

Conclusions and Recommendations for Monitoring Animal Movement

1. Monitoring animal movement by scoring the changes in signal strength resulting from the number of changes in orientation between coils of an implanted telemeter and the receiving antennae appears to provide valid data for the determination of circadian periodicity in pocket mice.

2. The technique can be very sensitive reflecting very slight changes in telemeter orientation. As a consequence it can be used to determine respiratory rate when the animal is at rest. This characteristic can be a drawback to the measurement of gross motor activity since the detection system cannot differentiate between signals resulting from gross movement and those resulting from respiration. For the detection of gross motor activity this problem is avoided by adjusting the detector sensitivity to respond only to relatively large changes in signal strength.

3. As a consequence of item (2) the proposed method of monitoring activity places some constraint on the cage/antennae system in which the studies are conducted. For example if a large and/or inefficient cage/antennae system is used the receiver must be operated at high gain to minimize signal "drop-out." At high gain there are positions at which respiration may be detected as gross movement. At low gain respiration will not be detected but there are positions in the cage where signal "drop-out" may occur.

4. The proposed method of monitoring animal movement has been incorporated on developmental models of space flight experiment hardware. Meaningful biological data were collected (Contract NAS2-5093).

5. It is specifically recommended that the proposed method of monitoring animal movement be implemented in experiment hardware for experiment MSFEB S-071.

Evaluation of a Heart Rate, Body Temperature and Animal Movement Telemetry System for Circadian Periodicity Studies

Accomplishment of this task was dependent upon the availability of a suitable telemeter. Prototype telemeters which monitored heart rate, body temperature, and animal movement were initially implanted in Swiss-Webster white mice to test surgical techniques and telemeter performance. On the basis of reasonable success with instrumented white mice a study was begun using pocket mice.

(1) Telemeter Development

Encapsulant effectiveness was greatly increased by applying a silicone sealant between the epoxy coating of the circuitry and the final wax coating of the total unit. The occurrence of electrode breaks at the point where the electrode penetrates the wax coating is also greatly reduced.

The use of electrode material other than stainless steel braided wire has not been adequately tested. A platinum-iridium alloy was tested and found adequate although undesirably rigid. The low incidence of electrode breaks in braided stainless steel suggests that new electrode material may not be necessary.

Proper quality control emphasizing assembly techniques which reduce strain on electrode leads and enhance encapsulant effectiveness; coupled with improved surgical techniques has increased the nominal life of the implanted heart-rate telemeter from approximately 30 days to approximately 90 days. It is anticipated that the nominal life will increase to at least 180 days by incorporation of a newly available battery.

(2) Surgical Techniques

Methods of telemeter implant including location of the telemeter and placement of electrodes has been standardized. An incision is made to the left of the midline penetrating the abdominal cavity. The sterilized telemeter is slipped into the abdominal cavity with care not to occlude the intestine and gently worked to a ventral position between the abdominal wall and the intestine. One electrode is sutured to the dorsal abdominal wall and the other electrode is led out of the abdominal cavity at the

anterior of the incision. The incision is sutured and the electrode protruding from it is led under the skin in a loose curve and sutured in the vicinity of the sternum which has been exposed by a second small incision.

A mortality of approximately 12% has been observed over the last six months directly attributable to the presence of the telemeter. In these cases the most common cause of death appeared to be poisoning resulting from battery leakage and rupture of the telemeter encapsulant. Improved encapsulation technique has greatly reduced this kind of mortality.

A mortality rate of 25% within 24 hours of telemeter implant was experienced during one series of implants which was attributable directly to surgical trauma. Surgical techniques, however, were the same as used both in earlier and subsequent periods.

(3) Biological Compatibility of Implanted Telemeters

Except in cases in which battery leakage and encapsulant rupture cause poisoning the biological compatibility of the telemeter implant has been exceptionally good. Mortality resulting from intestinal occlusion has been observed but is rare. Telemeters which have ceased to function have been left in animals for as long as five months. These animals when sacrificed showed no pathological effect of the long term implant.

Three implanted animals were monitored in developmental experiment hardware for two weeks, removed from the hardware, placed in small cages (3 x 3 x 4 inches), subjected to a range of sinusoidal vibration (Table II), removed from their cages, and replaced in the developmental experiment hardware. Visual inspection of the animals revealed no overt injury and subsequent monitoring failed to show any degradation in the quality of telemetered data. Ten days following vibration one animal died of what was assumed to be natural causes.

TABLE II

Sinusoidal Vibration Imposed on Three Instrumented Pocket Mice

Axis	Frequency CPS	Duration Minutes	Level G O-Peak	Sweep Rate
Lateral	10-19	0.46	2	1 Octave per min
	19-25	0.20	4.5	
	25-50	0.5	3	
	50-150	0.8	8	
	150-250	0.4	4	
	250-400	0.34	4.8	
	400-2000	1.17	5	

(4) Heart Rate Data

During days in which Perognathus parvus expresses torpor (T_A 20° C) the heart rate may vary from 600 beats per minute (bpm) to 70 bpm. However the heart rate is extremely arrhythmic compared to a Swiss-Webster mouse. It was initially planned to integrate the heart rate over a 10 second period and sample one each ten minute period. Heart rate data collected in this manner however are confused by the arrhythmicity and are of doubtful value to circadian periodicity studies. The heart rate was integrated for 20 seconds and sampled once each 10 minutes in the developmental experiment hardware (Figure 5). The quality of the data improved but suffered from the same inadequacies during periods in which the animal was normothermic. In laboratory equipment the heart rate was integrated over a 10 minute period and produced a near mirror image of the daily fluctuation in body temperature (Figure 6). A reasonable compromise will probably require that heart rate be integrated over a 1 to 5 minute period in the hardware to implement experiment MSFEB S-071. Resolution of this question is under study.

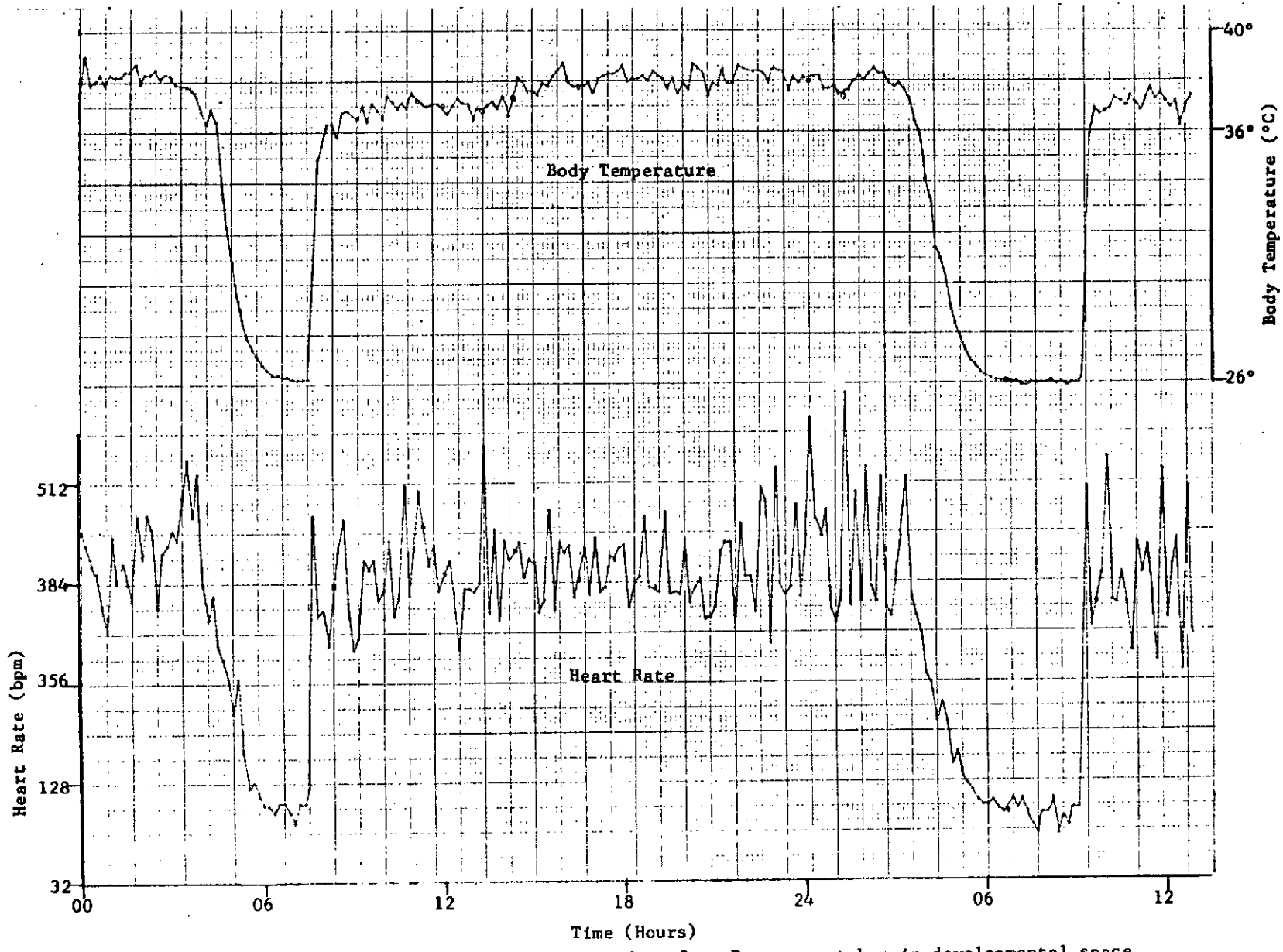

Figure 5. Comparative temperature and heart rate data from P. parvus taken in developmental space experiment hardware; temperature sampled 136 msec/10 min and heart rate sampled 19 sec/10 min.

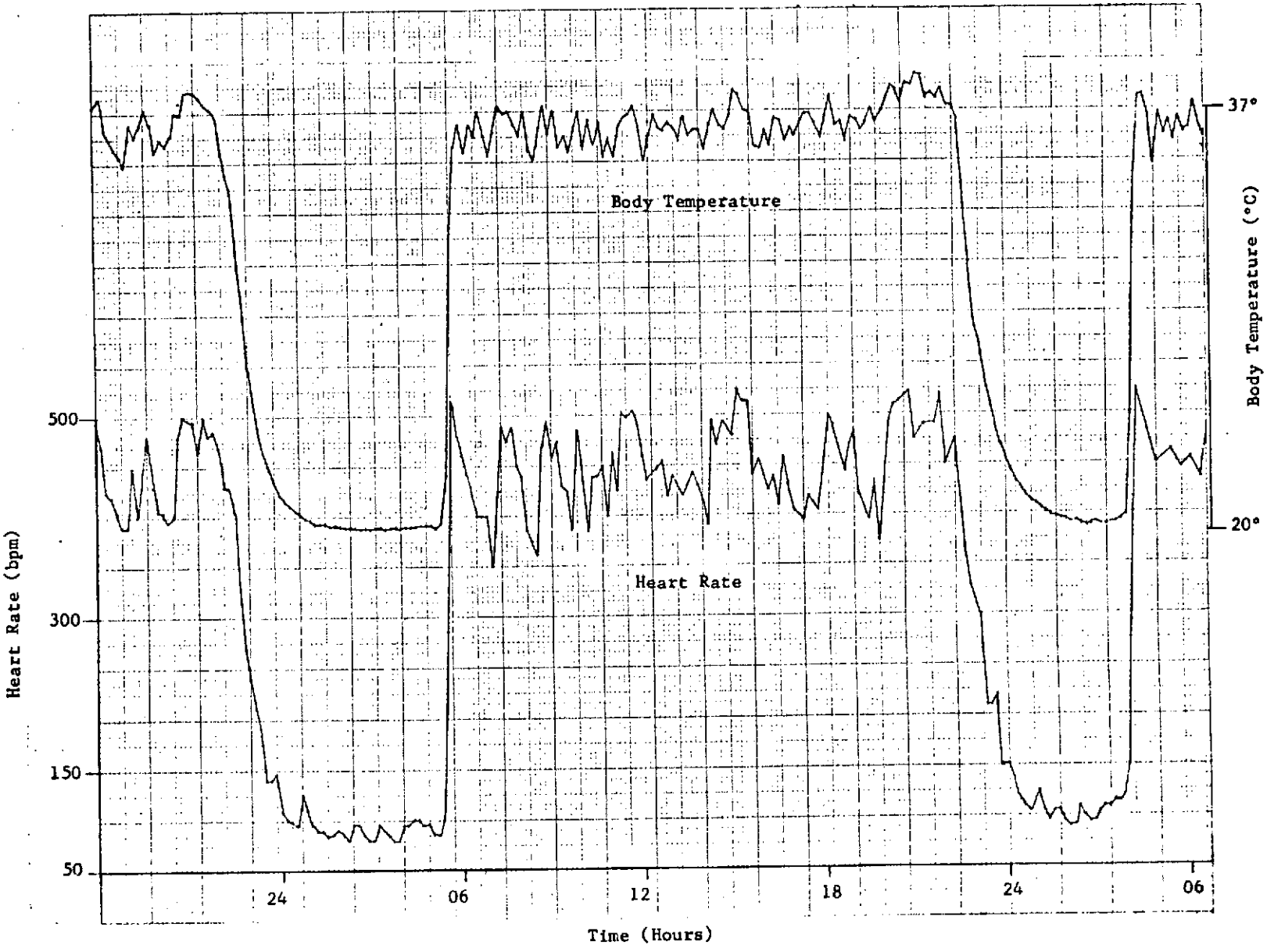

Figure 6. Comparative heart rate and temperature data of P. parvus, heart rate integrated for 10 min/10 min temperature sampled for 10 sec/10 min.

Determination of the Degree of Coupling Between the Circadian Periods of Heart Rate, Body Temperature, and Animal Activity

Development problems associated with the heart rate telemeter and support of Contract NAS2-5093 precluded collection of meaningful circadian periodicity data. In preparation for such studies however, a computer program to analyse circadian periodicity data was established. The program determines length of circadian period by autocorrelation, and as appropriate time of arousal from torpor, and provides power spectra and cross correlations. None of these statistical tools are unique but they include statistical methods most commonly utilized in the literature dealing with circadian period.

It is the intention that heart rate, body temperature and animal activity data be collected simultaneously from individual pocket mice and the data analysed not only by autocorrelation, etc., but also by special programs developed at Princeton University and University of Minnesota. The comparison should permit selection of the most appropriate method of analysis for experiment S-071. This research will be undertaken assuming renewal of this contract.

Support of NASA Contract NAS2-5093

Efforts under this contract provided instrumented pocket mice to engineers designing experiment hardware for experiment MSFEB S-071 and provided specific recommendations regarding experiment implementation. These recommendations are reflected in the Experiment Requirements Catalog prepared under Contract NAS2-5093.

CONCLUSIONS AND RECOMMENDATIONS

1. The feasibility and practicality of monitoring heart rate, body temperature, and animal movement by means of a single telemeter implant in pocket mice has been demonstrated.

2. The value of monitoring all three parameters remains to be demonstrated by experiments presently in progress.

3. A telemeter with which to study the circadian periodicity of heart rate, body temperature and animal activity is developed but has too short a life (2-3 months) to implement MSFEB S-071. The availability of a new battery with increased milliamp hour rating and improved seals may increase the useful life of the telemeter to six months or better.

4. It is recommended that current research be continued utilizing telemeters with improved batteries to verify the reliability of the animal/telemeter preparation for long term studies (3-6 months); and to collect quantitative biological data amenable to the study of circadian periodicity: such data to be collected in a manner to be useful in interpretation of experiment MSFEB S-071.

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APPENDIX B

Biotelemeter Specifications

Extracted from Report of Progress

1 April 1969 through 30 June 1969

TECHNICAL DISCUSSION

Background

Body temperature as a function of time is the primary datum required for the study of the stability of the circadian system of pocket mice in space. This is the parameter which has been intensively studied in the laboratory (NASr-91; NASw-812); documented in the literature; verified at Princeton; and ultimately led to the submission and acceptance of an orbital experiment (MSFEB S-071) scheduled for implementation during the Apollo Applications Program. Experiment hardware for execution of MSFEB S-071 has been completed under Contract NAS2-5093. Improved design and testing of a telemeter which will monitor heart rate as well as body temperature and animal movement has been initiated under Contract NAS2-5037 and is still in progress.

At this point in time, and in the context of the objectives of Experiment S-071, three alternative experimental designs are feasible.

1. Monitoring of body temperature alone in Perognathus longimembris.
2. Monitoring of body temperature and animal movement in Perognathus longimembris.
3. Monitoring of body temperature, heart rate, and animal movement in a larger species of pocket mouse such as Perognathus parvus.

None of these options significantly affect the design of hardware defined for execution of MSFEB S-071.

The third experiment option is preferred but its selection is dependent on demonstrating (1) the reliability of a body temperature/heart rate telemeter, and (2) the scientific value of the data collected with it. Demonstration of these two points is the prime objective of this contract.

Temperature Telemeter Circuit Design

The basic circuit is a relaxation or "squegging" oscillator type and is illustrated in Figure 1. This circuit may be described as being

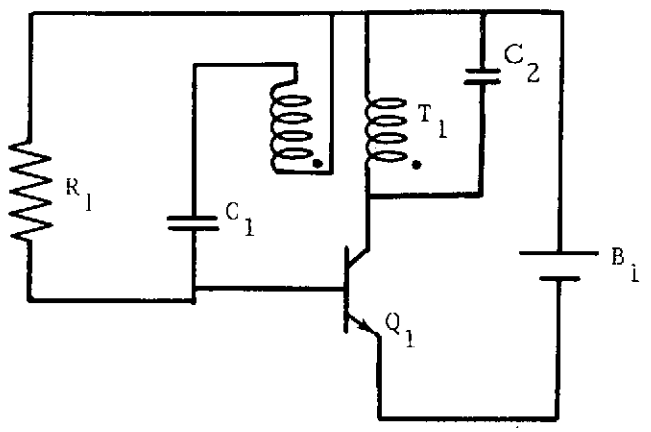

Fig. 1 Temperature Telemeter Circuit

PARTS LIST

Temperature Telemeter

B_1	Y1404 Mercury Battery Union Carbide
Q_1	NS 6214 Transistor National Semiconductor
R_1	1 Meg ohm Thermistor #44015 YSI - Components Division
C_1	.0033 μf. ± 20% Capacitor RU20-332-5 (black) Components Inc.
C_2	500 pf ± 10% Capacitor SCP-38D 501K (red) Cal-R Inc.
T_1	R.F. Transformer (600 KC) 100 T double wrap #44 enameled covered wire

intermediate between the more conventional LC oscillator and the blocking oscillator. If the capacitance-resistance (CR) combination controlling the base bias is increased, in the LC (sine wave) oscillator, the time required for the bias to again adjust itself to the small reduction in the total loop gain becomes longer until a point is reached where the oscillations decay spontaneously. If the total loop gain is now increased by increased feedback, the amplitudes of the oscillations themselves become oscillatory. As in the previous cycle, the oscillation again decays, the base bias leaks away at a rate determined by the capacitor-resistor combination and strong oscillations start once more. Oscillations build up very rapidly, generating a bias which eventually rises to a level too high to maintain them, cutting the base off, and the oscillations again die away. This mode of operation is the so called "squegging" circuit. In such a circuit, if the capacitance C is held constant and the R value is a varing resistance sensor (thermistor), then the pulse rate or squegging frequency becomes a function of temperature.

Temperature/Heart Rate Telemeter Circuit Design

Another channel of information can be transmitted by frequency modulation of the pulse frequency of the basic temperature telemeter. This circuit is illustrated in Figure 2. Frequency modulation is accomplished by varying the R value in the basic temperature telemeter circuit with a transistor. This transistor varies the RC time constant and thus the pulse frequency. With a 1 millivolt ECG signal at the input the frequency modulation of the pulse frequency is on the order of 3%. Thus the average pulse frequency is indicative of temperature and with a suitable fm detector circuit the ECG and heart rate can be displayed.

The detected ECG signals of the pocket mouse (Perognathus parvus) is shown in Figure 3 and after smoothing in Figure 4.

Typical calibration curves of the temperature and the temperature/heart rate telemeters are shown in Figure 5. The envelope of the calibration curves of the two types of telemeters could be narrowed by more intensive screening of components.

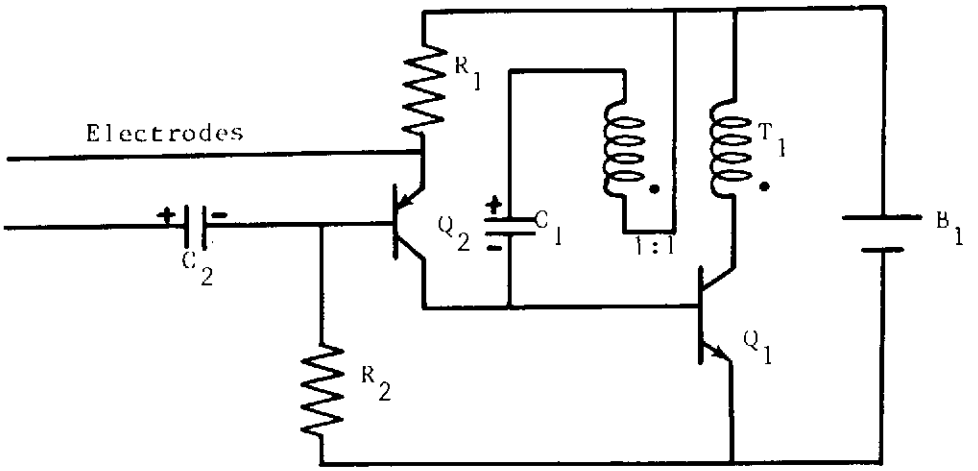

Fig. 2 Temperature/Heart Rate Telemeter Circuit

PARTS LIST

Temperature - ECG Telemeter

B_1	Y1404 Mercury Battery Union Carbide
Q_1	NS 6214 Transistor National Semiconductor
Q_2	NS 6201 Transistor National Semiconductor
R_1, R_2	1 Meg ohm Thermistor #44015 YSI - Components Division
C_1	.0022 μf. ± 20% Capacitor #C222 Components Inc.
C_2	1 μf. ± 20% Capacitor #C105 Components Inc.
T_1	R.F. Transformer (600 Kc) 136T Double wrap #44 enameled covered wire

Electrodes: Multistrand stainless steel wire. Silastic covered and filled. Statham Instruments

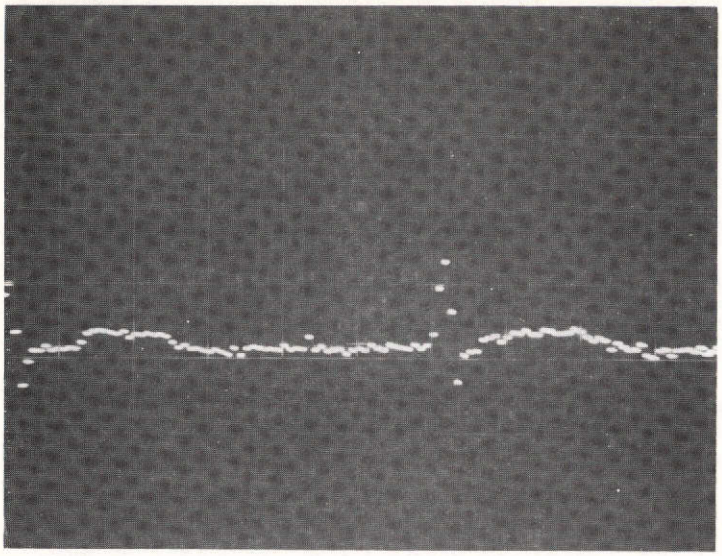

Fig. 3 Detected ECG Waveform of Pocket Mouse (P. parvus). Horizontal - 20 mill sec/cm. Vertical - .2 V/cm.

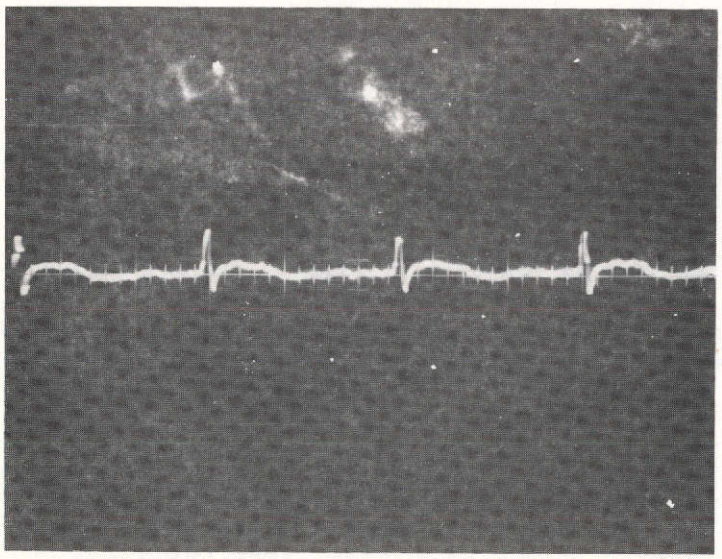

Fig. 4 ECG Waveform of Pocket Mouse (P. parvus) after smoothing. Horizontal - 50 mill sec/cm. Vertical - .5 V/cm.

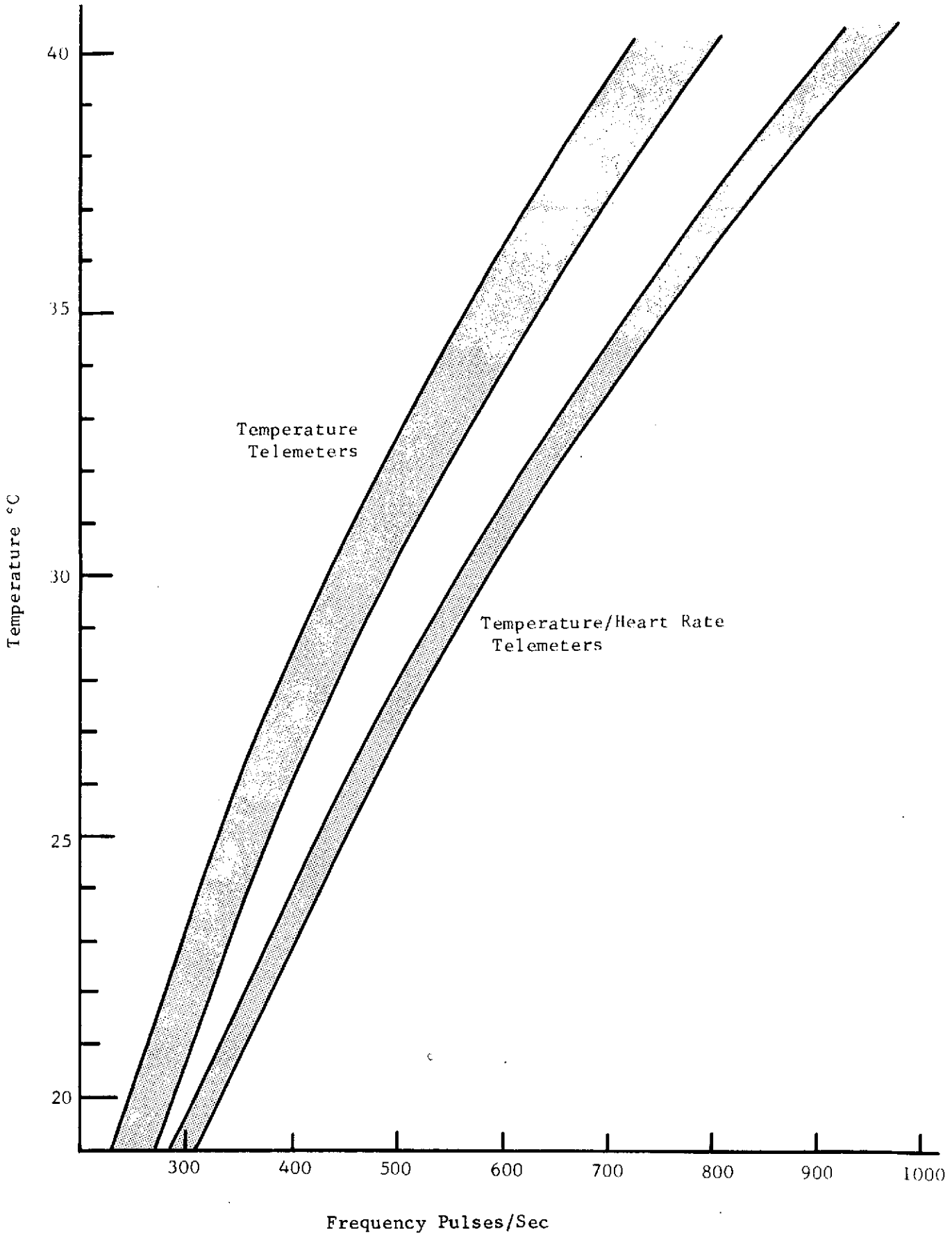

Fig. 5. Envelope of Calibration Curves of 13 Temperature Telemeters and 4 Temperature/Heart Rate Telemeters

Telemeter Component Selection

The components used in the fabrication of these biotelemetry transmitters are standard commercial items. In selecting the best grade of components available, the slight cost factor is far outweighed by the increased reliability. If close tolerance component values are used, the calibration curves of the various telemeters exhibit remarkable coincidence. Drift of component values induced by temperature variance does not pose a significant problem inasmuch as calibration is carried out on a total package basis and the entire telemeter is also exposed as an integral unit. In the implanted state accuracy and resolution remain unaffected.

(a) Power Source

Mercury cells were chosen for the energy source because of their small size and weight; efficiency in the temperature range of 20-40° C; stability of the terminal voltage after a short period of energy loss; and the stability of their internal impedence. The latter factors are particularly important since the simple telemeter circuit is sensitive to cell voltage level. Improved circuitry to remove this short coming results in significantly larger telemeters which are incompatible with very small animals.

Cell operational life can only be approximated from average current drain measurements since cell performance is not the same for steady state and pulsed conditions. For an average current level of 6×10^{-6} amps at 37° C, a 50 ma-hr cell should function for 8,333 hrs or about $11\frac{1}{2}$ months.

Mercury cells are designed generally for either low drain or high drain usage, depending on the barrier and the type of electrolyte used. In our case the average current drain ranges from 2 to 7×10^{-6} amperes with peak currents as high as 2×10^{-3} amperes.

The Mallory W-1 and the Eveready 312E were our initial choice because of their small size. After time consuming tests it was our

conclusion that these batteries could not provide the reliability required for long term implantation.

Union Carbide has developed a series of mercury oxide-zinc cells which are expected to be more reliable. The Y-1404 (50 ma-hr) is currently in use and initial results are encouraging.

(b) Transistors

Silicon NPN, micro transistor type WS-6214 has acceptable leakage characteristics and is modest in cost. Our experience with various types of micro-transistors, shows the WS-6214 to consistently operate with the lowest peak pulse current and thus lowest average current consistent with stable oscillations and a minimum of pulse to pulse jitter.

The silicon PNP, WS-6201 in the ECG telemeter is the only type we have tested in this particular circuit configuration and it appears to perform satisfactorily.

(c) Capacitors

Ultra-miniature solid tantalum capacitors were selected because of their extremely low leakage current and small size. Capacitance variation over the temperature range 20° C to 40° C is about 1%. Manufacturing tolerances of these units are $\pm$20% and this variation probably accounts for most of the spread in the telemeter calibration curves (Figure 5). A 0.002 μf. capacitor was used in the temperature/heart rate telemeters to increase the pulse rate per unit change in temperature to permit more samples of the ECG waveform at low temperature.

(d) Thermistor

The thermistor selected is the Yellow Springs Instrument type #44015. Performance characteristics of different units of the same type are within $\pm$ 0.5% of each other. This characteristic is of some importance in our design since we desired all telemeters of the same type to have similar calibration curves, similar duty cycles, etc.

(e) Tuned Transformer

The oscillation frequency (carrier R.f.) was selected to lie between 580 and 620 KC. At such frequencies signal transmission through tissue and body fluids are attenuated negligibly; and proximity capacitance effects on the oscillation are small enough to be neglected. Ancillary equipment, such as receivers, can be designed simply and economically.

Fabrication

The transmitter subassembly, consisting of the small component parts, is prefabricated, placed within the coil and wired in. Two gold-plated Kovar leads are brought out for micro-welding to the primary cell. The transmitter modules are mounted adjacent to and in the same plane as the battery. Transmitter geometry may be varied somewhat to conform better to size and shape of biological material to be monitored.

Encapsulation

Various epoxy and silicon materials have been tested and found to be permeable to body fluids when used as thin coatings. Present encapsulation procedures seal the circuitry with a thin coating of epoxy which is allowed "cure" for 12-24 hours before repeated dipping in a parafin-beeswax mixture. After the wax has hardened the units are trimmed to optimal size and shape and imperfections in the wax coating mended with a hot iron. The beeswax-parafin encapsulant has been used routinely over the last three years in our laboratory with no indication of tissue reaction.

The weight of the finished telemeter is a function of the thickness of the encapsulant. The temperature telemeter has a nominal weight of 0.8-1.0 grams and the temperature/heart rate telemeter a nominal weight of 1.3-1.5 grams.

Summary of Telemeter Characteristic

Specifications	Temperature	Temperature/Heart Rate
Excitation Potential	1.35 V dc	1.35 V dc
Frequency of Carrier	600 K Hz	.5 M Hz 600 K Hz
Average Current Drain	4 μa @ 25° C	4 μa @ 25° C
Pulse Modulation Frequency Range	200-1000 Hz	200-1000 Hz
Range	Approx. 1 ft	Approx. 1 ft
Temperature Range	20° - 40° C	20° - 40° C
Frequency Response	0 - .1 Hz	Temp 0 - .1 Hz ECG .5 - 100 Hz
Standard Cell (ASA)	M5 (50 ma/hr	M5 (50 ma/hr
Life (with M5 cell)	8000 hr	8000 hr
Modulation Coding	T° $\propto$ PRF	T° $\propto$ PRF ECG $\propto \wedge$ PRF
Size cm. (with M5 cell)	1.7 x 1 x .5 cm	1.7 x 1 x .5 cm
Volume	.85 cc	.85 cc
Weight	$\sim$ 1 grams	$\sim$ 1.5 grams
Encapsulation	Paraffin/beeswax	Paraffin/beeswax

APPENDIX C

PUBLICATIONS ON POCKET MICE RESULTING ALL OR IN PART FROM RESEARCH AT NORTHROP

*1. Lindberg, R. G. and P. Hayden: Temperature entrainment of the little pocket mouse, Perognathus longimembris. Submitted to Science, April 1974.

*2. Hayden, P. and R. G. Lindberg: Survival of laboratory reared pocket mice, Perognathus longimembris. Submitted to Journal of Mammalogy, January 1974.

*3. Lindberg, R. G., E. Halberg, F. Halberg, P. Hayden: Inversion of lighting regime alters acrophase relations of circadian rhythms in body temperature, heart rate, and movement in pocket mice. Space Life Sciences. 4:240-248, 1973.

*4. Lindberg, R. G., J. J. Gambino, and P. Hayden: Circadian periodicity of resistance to ionizing radiation in the pocket mouse. In Biochronometry, NAS Pub. Washington, D. C., pp. 169-185, 1971.

*5. Hayden, P. and R. G. Lindberg: Hemoglobin - oxygen affinity in pocket mice. Comp. Biochem. Physiol. 33:727-732, 1970.

*6. Hayden, P. and R. G. Lindberg: Hypoxia induced torpor in pocket mice (Genus Perognathus). Comp. Biochem. Physiol. 33:167-179, 1970.

*7. Hayden, P. and R. G. Lindberg: Circadian rhythm in mammalian body temperature entrained by cyclic pressure changes. Science, 162 (3885): 1288-1289, 1969.

8. Lindberg, R. G.: Feasibility study for conducting biological experiments aboard a Pioneer spacecraft. NASA CR 73178, 1968.

9. Chew, R. M., R. G. Lindberg and P. Hayden: Temperature regulation in the little pocket mouse (Perognathus longimembris). Comp. Biochem. Physiol., 21:487-505, 1967.

10. Lindberg, R. G.: Hibernation in the space age. Mammalian Hibernation III, pp. 439-444. Ed. K. C. Fisher et al., Aliver and Boyd Ltd. (1967).

11. Hayden, P., and J. J. Gambino: Growth and development of the little pocket mouse Perognathus longimembris. Growth 30:187-197, 1966.

2. Hayden, P., J. J. Gambino, R. G. Lindberg: Laboratory breeding of the little pocket mouse (Perognathus longimembris). J. Mamm. 47:412-423, 1966.

3. Gambino, J. J., R. G. Lindberg and P. Hayden: A search for mechanisms of radiation resistance in pocket mice. Rad. Res. 26:305-317, 1965.

4. Chew, R. M., R. G. Lindberg, P. Hayden: Circadian rhythm of metabolic rate in pocket mice. J. Mammal. 46:464-477, 1965.

5. Towner, J. W.: The effect of radioactive fallout at the Nevada test site on the Chromosomes of the pocket mouse. Health Physics 11:1569-1571, 1965.

6. Lindberg, R. G., G. J. DeBuono and M. M. Anderson: Animal temperature sensing for studying the effects of prolonged orbital flight on circadian rhythms of pocket mice. J. Spacecraft and Rockets 2:986-988, 1965.

7. Gambino, J. J. and R. G. Lindberg: Response of the pocket mouse to ionizing radiation. Rad. Res. 22(4):586-597 (1964).

[c]Published in course of Contract NAS2-5037.

APPENDIX D

Response of The Pocket Mouse To Chronic Acceleration

Extracted from Report of Progress

1 April 1971 through 30 June 1971

TECHNICAL DISCUSSION

RESPONSE OF POCKET MICE TO CHRONIC CENTRIFUGATION

Objective

The purpose of this experiment is to determine whether chronic centrifugation will affect either the precision or length of the free-running circadian period of body temperature in the little pocket mouse, Perognathus longimembris. The results of this experiment are intended to provide some basis for predicting the stability of the circadian system under conditions of weightlessness.

Experiment Rationale

A prime objective of MSFEB Experiment S-071 is to study the stability of the circadian system under conditions of weightlessness. This objective was recently reviewed and endorsed by a "Review Conference on Inflight Chronobiology Experiments" convened by NASA/OSSA on 25 September 1970. A recommendation resulting from the conference was that studies should be conducted to determine if circadian organization is influenced by chronic acceleration greater than 1 g. If circadian organization is influenced, it may be possible to extrapolate toward zero acceleration to predict possible effects of weightlessness.

Experiment Method

Twenty pocket mice were implanted with temperature sensing telemeters representative of those manufactured for Experiment S-071 by Northrop Electronics Division. Five days post surgery the implanted animals were placed in the HU/LMU (Holding Unit/Laboratory Monitoring Unit) and monitored for ten days at a cage ambient temperature of 20° C. On the tenth day, in a dark room illuminated with a ruby red photographic safe light, six animals showing best evidence of daily torpor were removed from the HU/LMU, placed in light tight containers and transferred by air to NASA/ARC. At NASA/ARC, again in the dark under red light, the animals

were transferred to simulated flight hardware (LTM), and the hardware subsequently mounted on the 20 g centrifuge (Bldg. N 221-A). Each animal was provided 50 grams of air dried seeds. The entire transfer, from the Northrop laboratory to mounting on the NASA/ARC centrifuge, took approximately five hours.

A proportional temperature controller and heating rod were located at the air intake of the simulated flight hardware to heat ventilating air, as necessary. The experiment was conducted at a cage ambient air temperature of $20^{\circ} \pm 0.5^{\circ}$ C.

Body temperature of each mouse, one ambient cage air temperature, and one accelerometer measurement were recorded continuously on an eight channel analog recorder.

Following checkout of all equipment the centrifuge was turned on, gradually increasing to 7 rpm, to produce a 1.1 g vector through the floor of the mouse cage. The intent was to monitor the animals for three weeks at 1.1 g and then to increase to 2.2 g for three weeks. However, on the twentieth day, the centrifuge began a series of stops and starts (including one 3 g spike) to which some of the animals apparently reacted. As a consequence, the 1.1 g force was continued for 26 days in hope that the operational problem would be remedied. The scheduling of centrifuge time and the possibility of the animals running out of food forced a decision to increase the g level to 2.2 g even though it was questionable whether all animals had restabilized from the period of intermittent stops and starts.

After seventeen days at 2.2 g, the pattern of torpor of the remaining animals suggested that they might be having difficulty feeding or handling the increased dead weight of the telemeters. The g level was subsequently reduced to 1.6 g for another eight days before the experiment was terminated.

Results

Plots of entry and arousal from torpor on successive days of continuous centrifugation are presented for each pocket mouse in Figures 1 - 6. Plot of arousal from torpor only (phase reference point) on successive days,

together with a history of disturbances due to handling, travel, and centrifugation, are presented for each mouse in Figures 1A - 6A. The two kinds of plots taken together (i.e., Figure 1 and Figure 1A) give a good summary of the thermoregulatory behavior in each mouse and the possible effects of disturbances on the free-running circadian period (τ_{FR}).

The telemeter failed in the mouse depicted in Figures 4, 4A and there is some question as to whether the last two times of arousal are biologically real. The mouse in Figures 6, 6A died shortly after a 3 g acceleration spike and it is assumed that the animal was injured in some way by the incident: most probably internal injuries were produced by the telemeter.

The animals shown in Figures 1, 3 and 4 showed an average decrease in normothermic temperature of 2-3° C for 3 - 6 hours immediately following the transition from 1.1 g to 2.2. At termination of the experiment, only two animals were still alive. Food was present on all cages except for the animal shown in Figures 1, 1A.

Discussion

For convenience the data will be discussed below in terms of specific time periods: the time at Northrop in the HU/LMU; the time at 1.1 g; the time at 2.2 g; the time at 1.6 g.

(a) HU/LMU

Although 20 animals were placed in the HU/LMU, all had been recently implanted. After ten days only a few had begun expressing a daily torpor (which is not unusual). Scheduling of the centrifuge required that the experiment be initiated immediately. As a consequence, six animals were selected mainly on the basis of having expressed regular torpor rather than any detailed analysis of the characteristics of their τ_{FR}. It is a point of some interest that despite being handled, flown to San Jose, driven to Moffett Field, and again handled as they were placed in the experiment hardware, the τ_{FR} was relatively unaffected in five of the six animals.

(b) 1.1 g

All animals showed a reasonably good τ_{FR} at 1.1 g up until the time of intermittent stopping of the centrifuge. The animals in Figures 3 - 4 - 5 - 6 did not appear to respond to these stimuli. The animals in Figures 1 and 2 however, show a strong tendency for a shortening of the τ_{FR}. However, both of these latter animals show some tendency fo period shortening just prior to the stops and starts, and it is not clear that the interruptions alone account for their behavior.

Only the animal in Figure 6 showed any response to the 3 g spike. Since it died shortly thereafter, it is assumed that it was injured. Since numerous implanted pocket mice have survived more severe acceleration profiles in earlier testing, it is assumed that this mishap is in the category of a "freak" accident. Note the shortening and improved precision of τ_{FR} in Figure 6 immediately following the 3 g spike. This kind of pattern has been observed frequently in pocket mice nearing death (see Figure 2A - 5A). As can be seen in Figure 3A, however, the correlation is not absolute. The pattern can also be induced in animals if the food supply is removed.

(c) 2.2 g

A reduction in body temperature immediately following transition to a higher g level has been reported as common for white rats (J. Oyama NASA/ARC Personnel Communication). In this experiment, however, only three animals showed a depression of body temperature and then for a relatively short period (3 - 6 hours). Body temperature in P. longimembris is closely coupled to degree of activity. A variatio of 3 - 5° C between body temperature of animals during rest and activity is not uncommon. It is our interpretation that the three animals showing the depression at the time of the transition may have experienced some difficulty in warming simply because they found it difficult to move or orient in the 2.2 g environment. Animals which did not show the depression may have been more disturbed by the transition and exerted more energy in trying to adapt to it. The effect at best was transitory.

It is difficult to make a clear determination of the effect of the transition from 1.1 to 2.2 g, since on the day following the transition the centrifuge stopped for about one hour. It can be argued from the data that the immediate effects following the transition resulted as much from the one hour interruption as the transition, itself.

The animal in Figure 1 did not express torpor for three days following the transition. The first torpor following the transition suggests that phase advance has occurred with some τ_{FR} shortening. However, by the ninth day the period has begun to lengthen and approximates the τ_{FR} expressed in the early part of the 1.1 g regimen. We would predict that, had this particular animal been left at 2.2 g for the duration of the experiment, the data would not look different from data presented in Figures 1, 1A at 1.6 g.

The animal in Figure 2 did not express torpor on the day following the transition; began to reestablish the τ_{FR} expressed at 1.1 g; phase shifted for no apparent reason and on the ninth or tenth day following the transition, went into a typical pattern of a dying mouse and failed to arouse twelve days after the transition.

The animal in Figure 3 was obviously the most disturbed by the 2.2 g environment. Periods of torpor are short intermittent and almost arhythmic. There is, however, a tendency toward lengthening of the τ_{FR}.

The animal in Figure 4 does not go torpid the day following the transition. On the second day, however, there is an apparent phase shift followed by a tendency to maintain the τ_{FR} established at 1.1 g. Whether the shift resulted from the transition from 1.1 to 2.2 g or to the subsequent stop and start of the centrifuge cannot be determined. On the seventh day following the transition, the period appears to lengthen. From earlier observations we are inclined to believe this phenomenon an artifact related to telemeter failure. At termination of the experiment the mouse was dead but time of death is unknown.

The animal in Figure 5 appeared relatively unaffected by the 2.2 g environment up until nine or ten days following the transition. At this time the duration of torpor increases and the τ_{FR} begins to shorten, once again a sign of a dying animal.

The animal in Figure 6 died shortly after the transition to 2.2 g, presumably of injuries received during the 1.1 g regimen.

(d) 1.6 g

The animal in Figure 1 did not express torpor on the day following the transition from 2.2 - 1.6 g, suggesting that it did sense the change in acceleration. The reduction to 1.6 g, however, had no effect on the τ_{FR}. On day 149 a loss of power to the experiment resulted in a reduction of ambient temperature for about one hour although the centrifuge rotation was unaffected. Ambient cage temperature could not be monitored during this period but it could not have dropped below the room ambient of 15° C. Coincidental with loss of temperature control is some variation in the time of arousal from torpor on subsequent days in Figure 1A. In view of the variation in time of arousal during the 1.1 g regimen, it is doubtful that loss of temperature control produced this effect. The shortening of the τ_{FR} and increased length of torpor during the last few days' experiment is characteristic of animals held without food. At termination of the experiment the animal was alive but the food supply was exhausted.

The animal in Figure 3 responded to the transition to 1.6 g by almost immediately reestablishing a τ_{FR} similar to the one expressed at 1.1 g. Again the increased duration of torpor suggested an exhausted food supply. This animal did not show a response to the one hour loss of temperature control. At termination of the experiment the animal was alive with a very low supply of seeds.

Except for the animals in Figures 1 and 3, all were dead at termination of the experiment and all cages contained food. The reason for the high mortality is unknown. It is unlikely that starvation was the cause of death since food was available and durations of torpor

were not typical of starving animals. We are inclined to believe in retrospect that 2.2 g was too high an acceleration force to expect a 10 gram mouse with 1 gram dead weight telemeter to survive. The protocol should have gradually increased the g level from 1.1 g to 1.6 g to 2.0 g.

Conclusions

In our opinion this experiment has not provided data to support the premise that the τ_{FR} of body temperature is in any way dependent on g level. It is our interpretation that the imprecision of τ_{FR} after the first 30 days of the experiment are the result of intermittent stimuli resulting from stopping and starting the centrifuge and transitions from one g level to another, coupled with the inability of some animals to cope with the increased dead weight of the telemeter at high g levels.

It is our prediction that if the experiment was repeated, and free of operational anomalies, we would still find the τ_{FR} relatively unaffected at g levels which pocket mice implanted with telemeters could survive. It is doubtful that the resulting data could be extrapolated to a zero acceleration environment.

Recommendation

The question posed by the Review Conference on Inflight Chronobiology is still unanswered. In this perspective the experiment should be repeated.

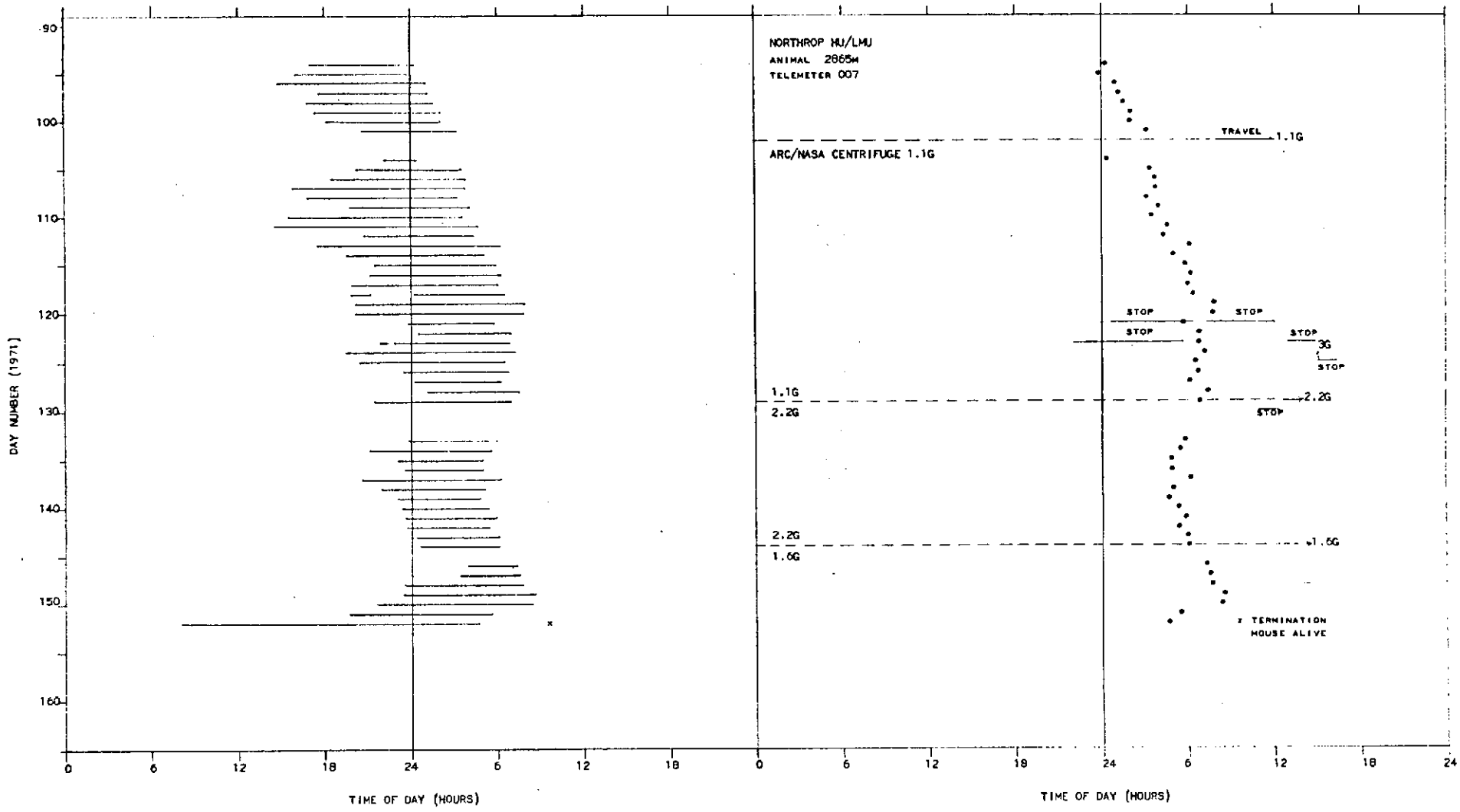

FIG. 1 - PLOT OF ENTRY AND AROUSAL FROM TORPOR ON SUCCESSIVE DAYS OF A POCKET MOUSE SUBJECT TO VARYING DEGREES OF CONTINUOUS CENTRIFUGATION. SOLID BAR INDICATES DURATION OF TORPOR. TIME AND DEGREE OF CENTRIFUGATION IS SHOWN IN FIGURE 1A.

FIG. 1A - TIME OF AROUSAL FROM TORPOR ON SUCCESSIVE DAYS OF ANIMAL IN FIGURE 1. SOLID BARS INDICATE DURATION OF PERTURBATIONS DUE TO TRAVEL AND STOPPING OF CENTRIFUGE.

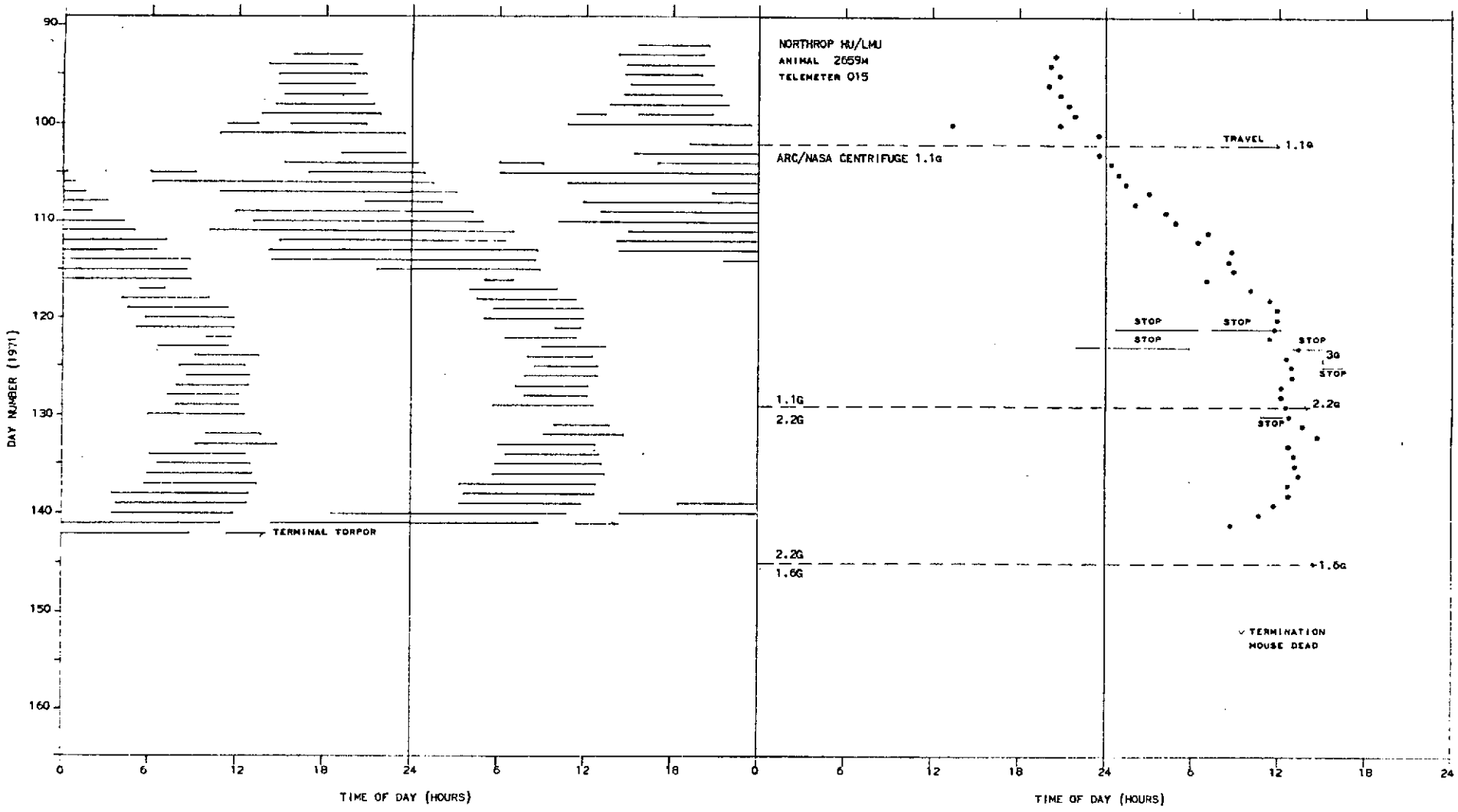

FIG. 2 - DOUBLE PLOT OF ENTRY AND AROUSAL FROM TORPOR ON SUCCESSIVE DAYS OF A POCKET MOUSE SUBJECT TO VARYING DEGREES OF CONTINUOUS CENTRIFUGATION. SOLID BAR INDICATES DURATION OF TORPOR. TIME AND DEGREE OF CENTRIFUGATION IS SHOWN IN FIGURE 1A.

FIG. 2A - TIME OF AROUSAL FROM TORPOR ON SUCCESSIVE DAYS OF ANIMAL IN FIGURE 2. SOLID BARS INDICATE DURATION OF PERTURBATIONS DUE TO TRAVEL AND STOPPING OF CENTRIFUGE.

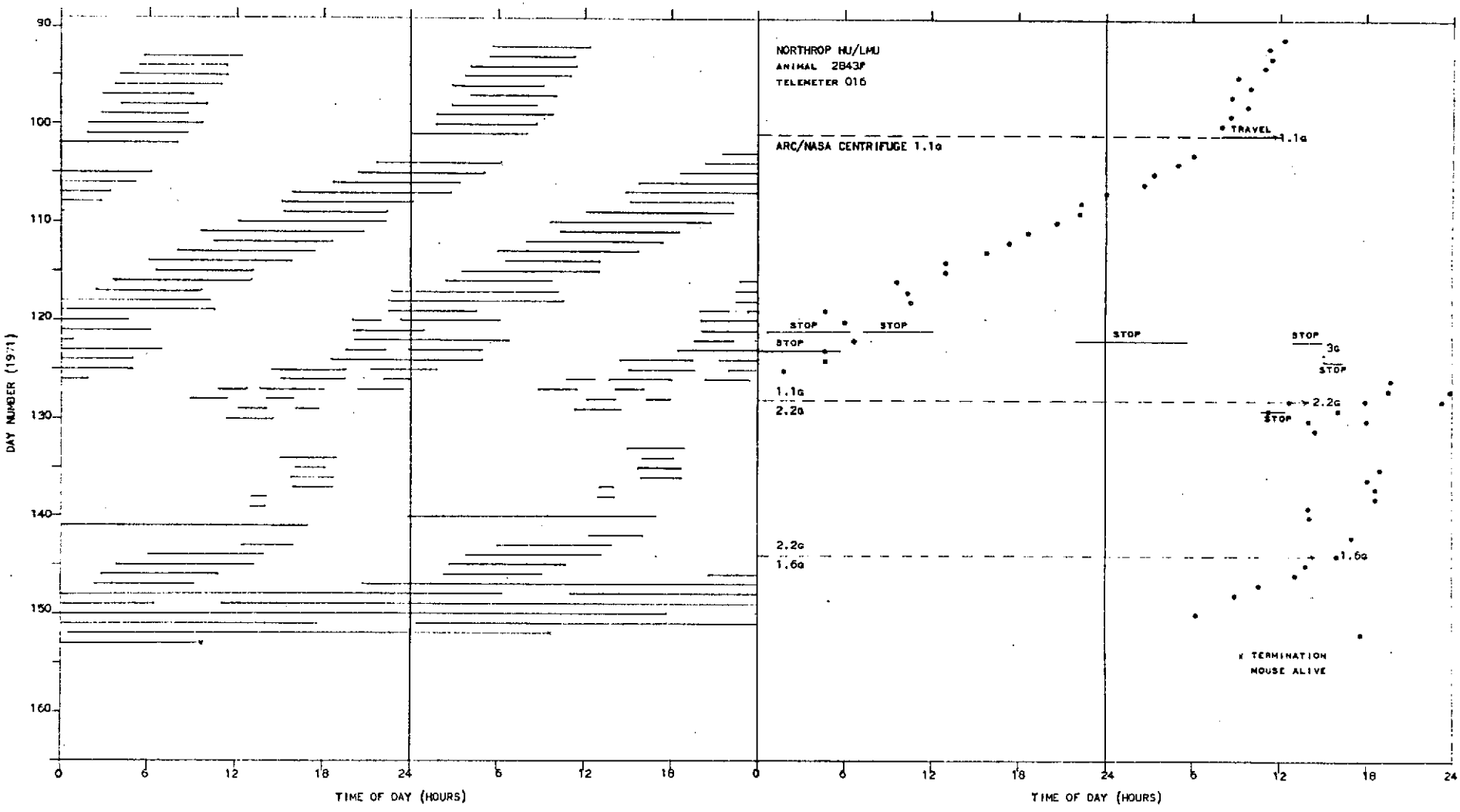

FIG. 3 - DOUBLE PLOT OF ENTRY AND AROUSAL FROM TORPOR ON SUCCESSIVE DAYS OF A POCKET MOUSE SUBJECT TO VARYING DEGREES OF CONTINUOUS CENTRIFUGATION. SOLID BAR INDICATES DURATION OF TORPOR. TIME AND DEGREE OF CENTRIFUGATION IS SHOWN IN FIGURE 3A.

FIG. 3A - TIME OF AROUSAL FROM TORPOR ON SUCCESSIVE DAYS OF ANIMAL IN FIGURE 3. SOLID BARS INDICATE DURATION OF PERTURBATIONS DUE TO TRAVEL AND STOPPING OF CENTRIFUGE.

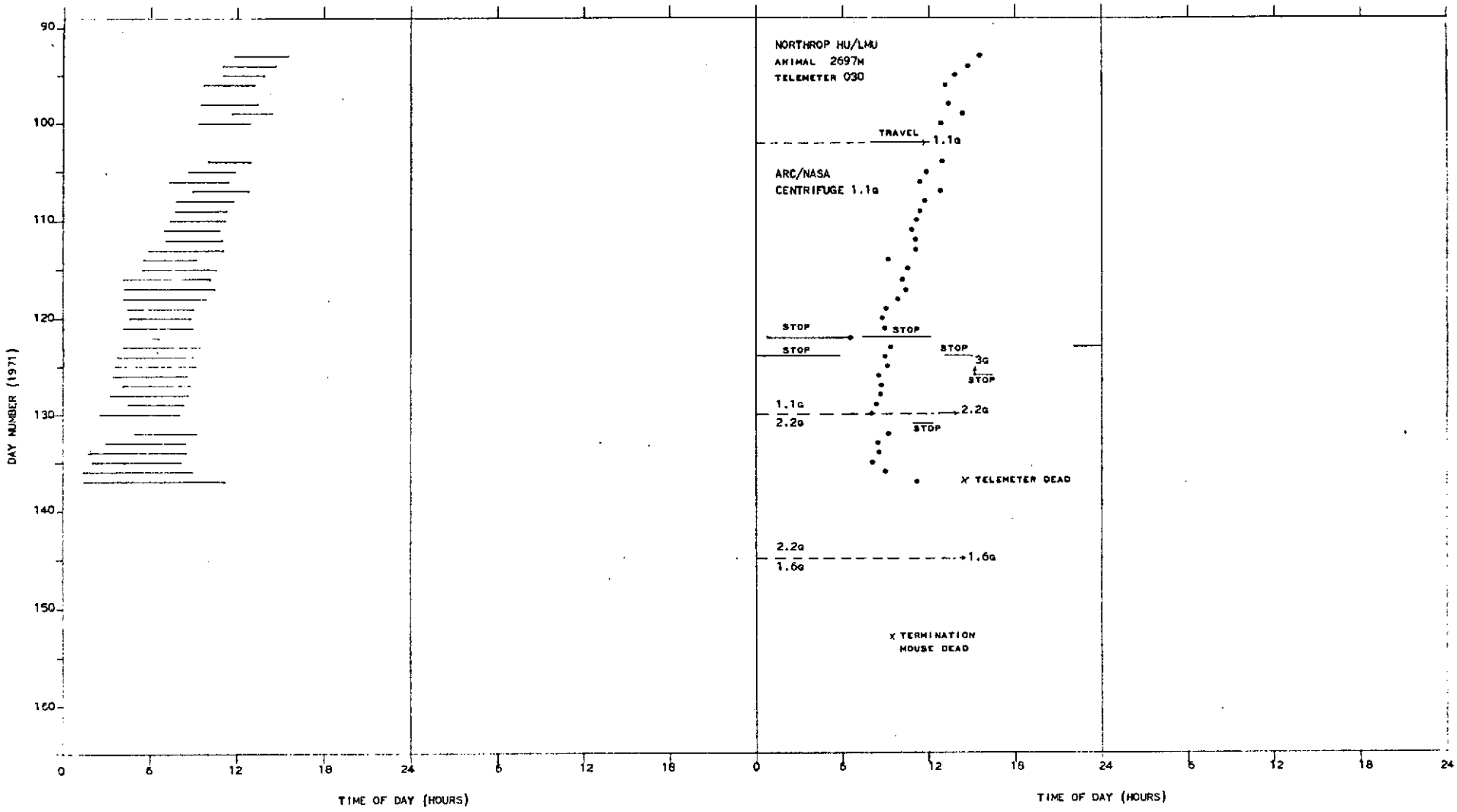

FIG. 4 - PLOT OF ENTRY AND AROUSAL FROM TORPOR ON SUCCESSIVE DAYS OF A POCKET MOUSE SUBJECT TO VARYING DEGREES OF CONTINUOUS CENTRIFUGATION. SOLID BAR INDICATES DURATION OF TORPOR. TIME AND DEGREE OF CENTRIFUGATION IS SHOWN IN FIGURE 4A.

FIG. 4A - TIME OF AROUSAL FROM TORPOR ON SUCCESSIVE DAYS OF ANIMAL IN FIGURE 4. SOLID BARS INDICATE DURATION OF PERTURBATIONS DUE TO TRAVEL AND STOPPING OF CENTRIFUGE.

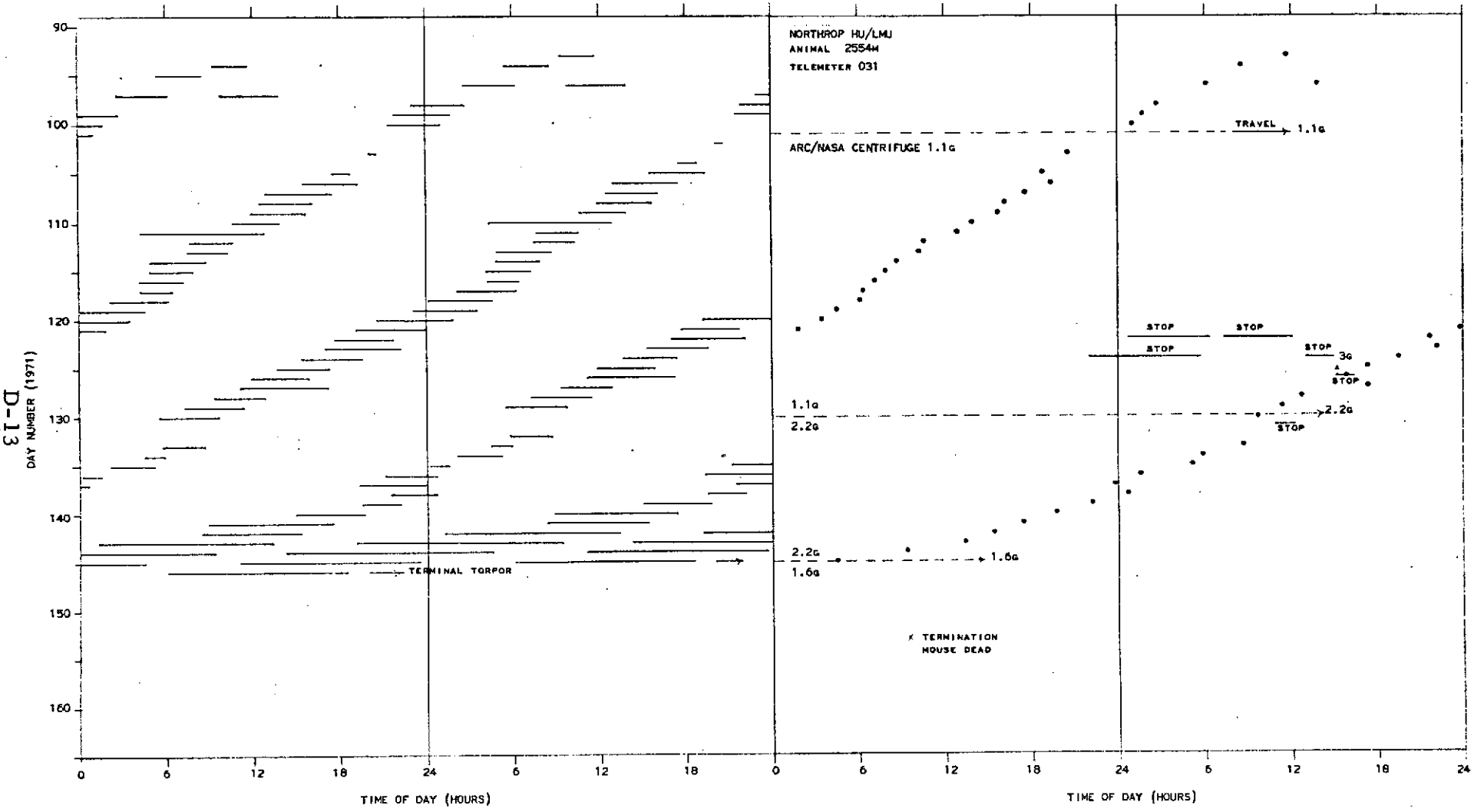

FIG. 5 - DOUBLE PLOT OF ENTRY AND AROUSAL FROM TORPOR ON SUCCESSIVE DAYS OF A POCKET MOUSE SUBJECT TO VARYING DEGREES OF CONTINUOUS CENTRIFUGATION. SOLID BAR INDICATES DURATION OF TORPOR. TIME AND DEGREE OF CENTRIFUGATION IS SHOWN IN FIGURE 5A.

FIG. 5A - TIME OF AROUSAL FROM TORPOR ON SUCCESSIVE DAYS OF ANIMAL IN FIGURE 5. SOLID BARS INDICATE DURATION OF PERTURBATIONS DUE TO TRAVEL AND STOPPING OF CENTRIFUGE.

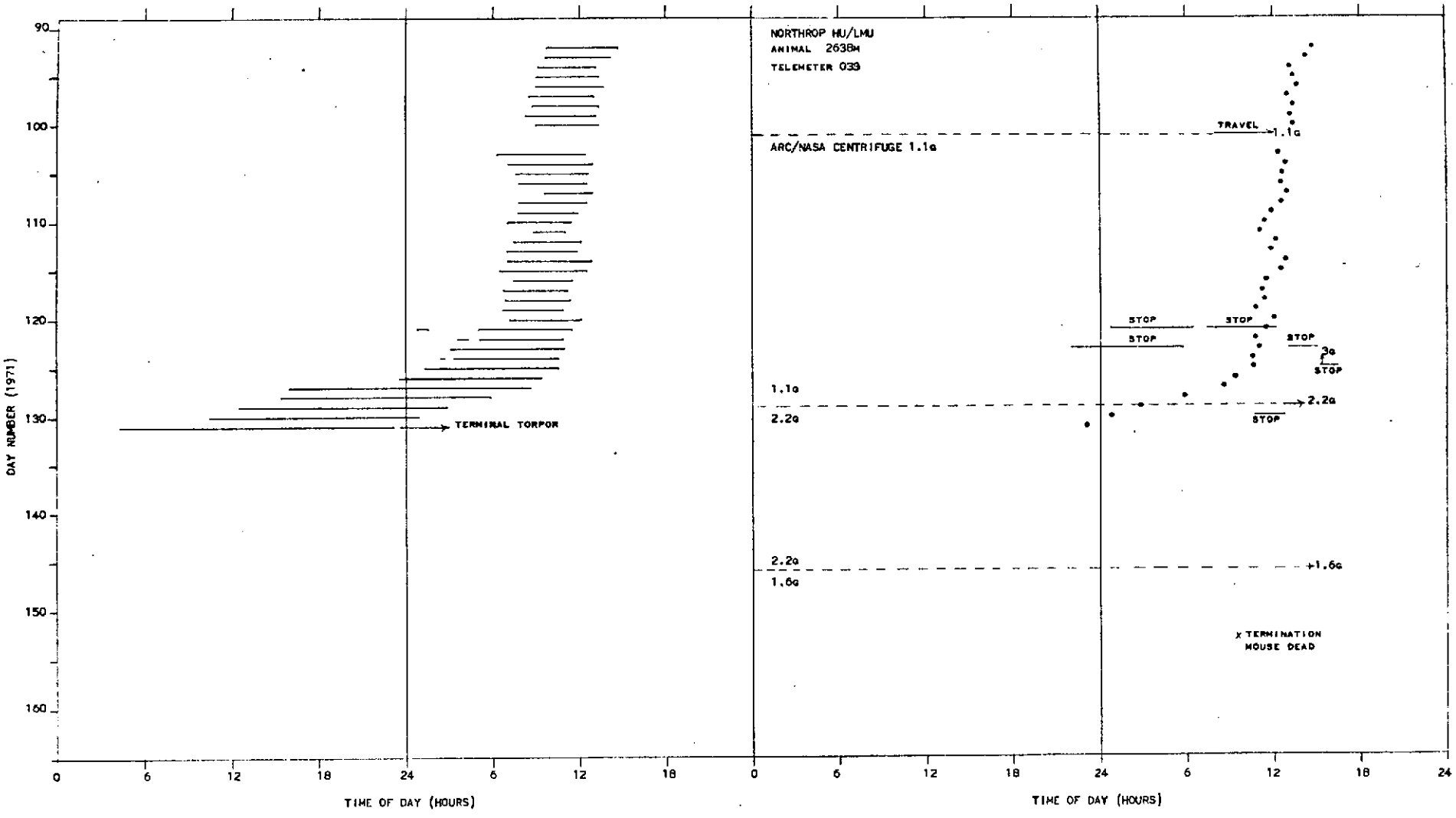

FIG. 6 - PLOT OF ENTRY AND AROUSAL FROM TORPOR ON SUCCESSIVE DAYS OF A POCKET MOUSE SUBJECT TO VARYING DEGREES OF CONTINUOUS CENTRIFUGATION. SOLID BAR INDICATES DURATION OF TORPOR. TIME AND DEGREE OF CENTRIFUGATION IS SHOWN IN FIGURE 6A.

FIG. 6A - TIME OF AROUSAL FROM TORPOR ON SUCCESSIVE DAYS OF ANIMAL IN FIGURE 6. SOLID BARS INDICATE DURATION OF PERTURBATIONS DUE TO TRAVEL AND STOPPING OF CENTRIFUGE.